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COEFFICIENTS OF DIGESTIBILITY OF THE CONSTITUENTS OF MILK AND THE BALANCE OF CALCIUM AND PHOSPHORUS IN CALVES ON A MILK DIET*

By

J. S. HUGHES AND H. W. CAVE

(From the Kansas Agricultural Experiment Station, Manhattan)

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IN CONNECTION with work on the influence of the feed received on the nutritive value of the milk produced, two calves were fed on a diet of milk alone. After these calves had been on this exclusive milk diet for a period of several months, a metabolism trial was run in which the coefficient of digestibility of the constituents of the milk and a mineral balance were determined. It is the purpose of this article to give the results of this digestion trial. The question of the deficiences of milk as an exclusive diet for calves will be discussed in another paper.

HANDLING PREVIOUS TO THE DIGESTION TRIAL

Two Holstein calves, No. 17 a male and No. 18 a female, were placed on a diet of milk alone at birth and were continued on this diet for a year or more. The amount of milk given varied from eight pounds of whole mixed herd milk daily at birth to 10 pounds of pasteurized skim milk and 15 pounds of whole mixed herd milk during the trial. For approximately the first six months whole milk alone was used, as much as 20 pounds per calf being fed. The milk was not fed *ad libitum* or according to standards but was limited to the consumption of milk by two calves on another phase of the experiment for which calves No. 17 and No. 18 were serving as controls. At times the amount of the digestible nutrients received by the calves was considerably below the minimum requirements for growing dairy cattle as given by Morrison (1) but in the main the true protein and net energy approximated the requirements as given by Armsby (2). At approximately 8 and 7½ months of age respectively, the digestion trial here discussed was conducted. During the period of the digestion trial the two

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calves were receiving 62 and 70 per cent respectively of the minimum requirements for total digestible nutrients as given by the Morrison standard. The calves were stabled in straw bedded stalls and were turned in a dry lot whenever the weather was suitable. They were given free access to water from the college mains while in the lot and were watered twice daily while in the barn. The calves were given access to salt frequently.

Except at feeding time the calves were muzzled with a muzzle shown in Figure 1, made from ¼ inch mesh hail screen with a sheet metal bottom and fitted to a leather halter. When a tendency was noted for the calves to work bedding material through the screen, a lining of ordinary screen wire and later of copper screen wire was inserted in the muzzles.

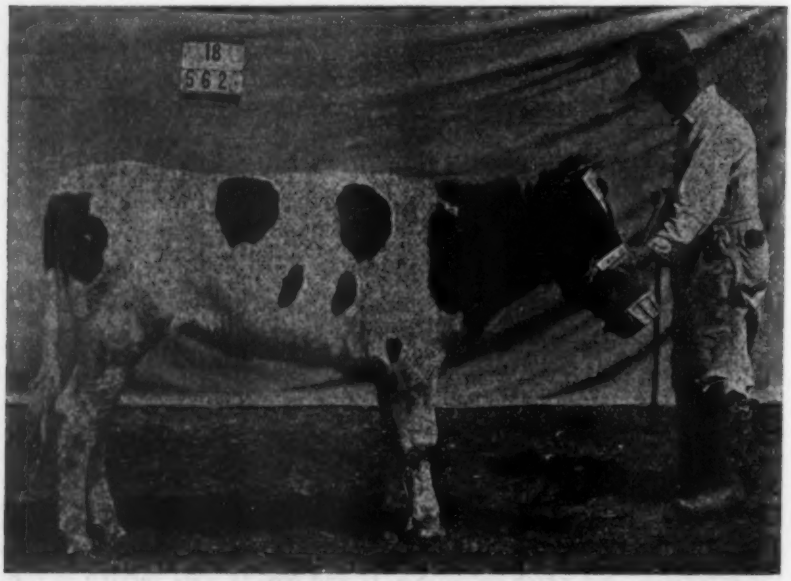

FIG. 1. Showing type of muzzle used.

On Monday and Tuesday of each week the calves were weighed and on Monday they were measured to determine growth, the following measurements being taken: height at withers, chest girth, barrel girth, width of hips, and width of pin bones. Figures 2 and 3 show that the calves at the time of the digestion trial were considerably below normal[1] in body weight.

[1] The normal used is the average weight and height of similar calves of comparative ages fed normally at the Kansas Agricultural Experiment Station.

At the same time they were approximately normal in height of withers as is shown in Figures 4 and 5. This would indicate normal growth of body framework but a lack of body fat and fleshing. Post mortem examination

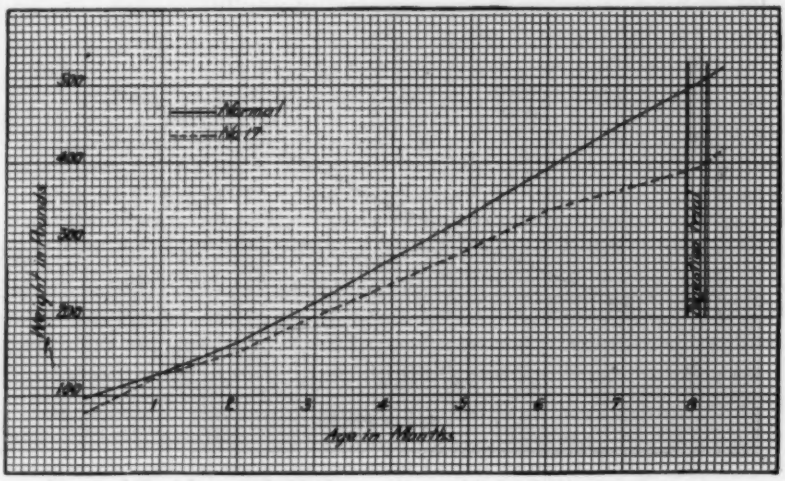

FIG. 2. Showing weight as compared with the normal for Holstein males.

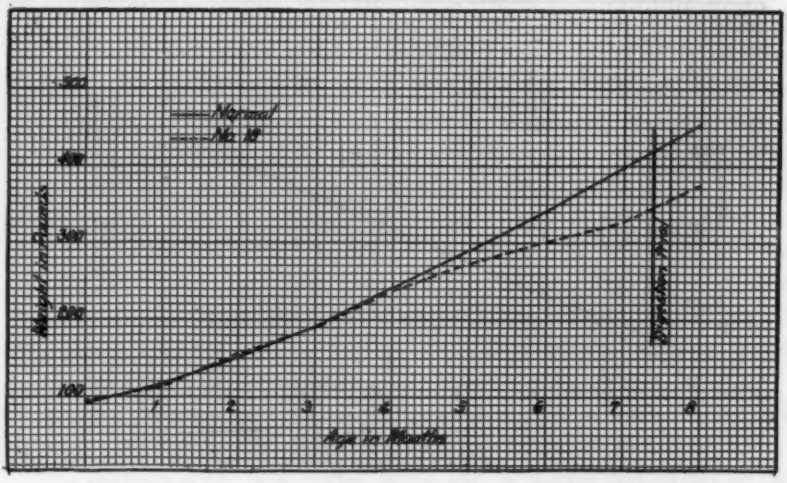

FIG. 3. Showing weight as compared with the normal for Holstein females.

of No. 17 and of other calves on an exclusive milk diet has shown a decided lack in size and tone of the organs of digestion.

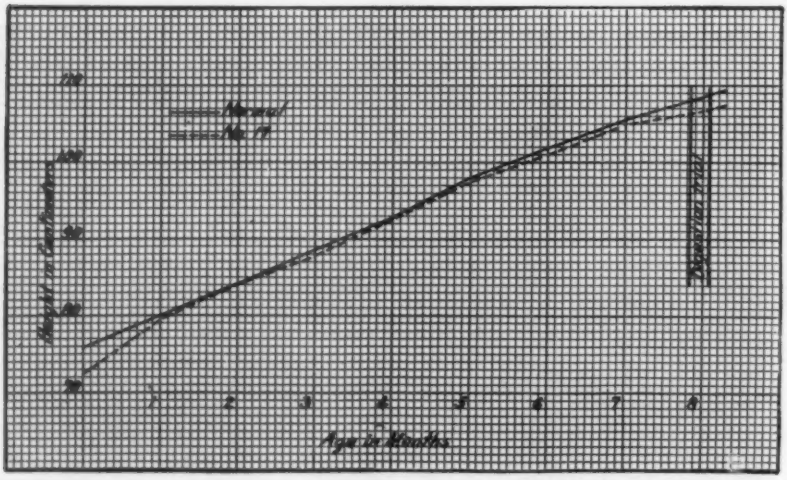

FIG. 4. Showing height of withers as compared with the normal for Holstein males.

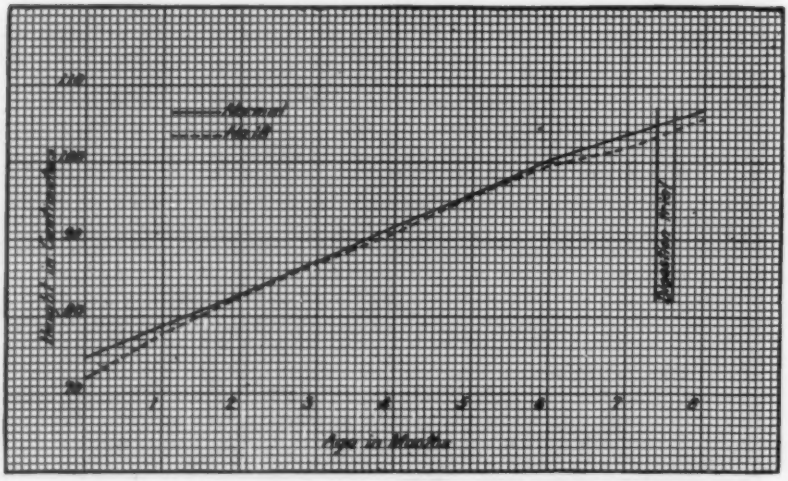

FIG. 5. Showing height of withers as compared with the normal for Holstein females.

Experimental Procedure

The calves were removed from the dairy barn to special stalls in the nutrition laboratory three days before the metabolism trial was begun, so they would become accustomed to the new surroundings prior to the collection of samples. As they were to continue on the same kind of milk they had been receiving, it was not necessary to run a long preliminary feeding period.

The milk used was a mixture of whole milk and centrifuged milk in the ratio of two-fifths of the former and three-fifths of the latter. Each calf was given twenty-five pounds a day. This was divided into two feeds, one-half being given in the morning and the other in the evening. At each feeding time a sufficient amount of whole and skimmed milk was mixed together to feed the two calves and to allow some for sampling. A 100 cc. sample was taken at each feeding and these were composited for the seven-day sample. After each feeding the calves were given all the hydrant water they would drink. The amount of water consumed by each calf was recorded so that the amount of calcium received in the water could be determined.

The collection of the urine and feces was cared for by two attendants who worked in six-hour shifts. The urine of the male calf (No. 17) was collected in the usual manner by means of a rubber funnel, held in place by a suitable harness, and a rubber tube running through the floor to a bottle. The urine of the female calf (No. 18) was caught in a suitable dipper and placed in a bottle. In neither case was there any appreciable loss of material. The twenty-four hour sample from each calf was weighed, thoroughly mixed and an aliquot of one-twentieth taken for analysis. These aliquots were composited for the seven days, chloroform being used as a preservative. The feces were caught in dippers and transferred to wide-mouth bottles containing alcohol. The amount of feces, which was voided on the average of once during the twenty-four hours, was very slight. They were light in color, fairly solid in consistency, and had very little odor. At the end of the seven-day period the alcohol was evaporated off and the residue was brought to dryness in an oven heated to 100 degrees C. The entire sample for each calf, 374.2 grams for No. 17 and 396.6 grams for No. 18 was then ground and sampled for analysis. The amounts of milk and water consumed and of urine and feces voided are shown in Table I.

Calf No. 18 consumed very little water as compared with No. 17 due perhaps to the change of environment during the metabolism trial.

The analyses of the milk, water, urine, and feces were carried out by the

TABLE I

GRAMS OF MILK AND WATER CONSUMED, AND URINE AND FECES VOIDED DURING SEVEN-DAY METABOLISM TRIAL

Calf No.	Grams of milk consumed	Grams of water consumed	Grams of feces voided (dry weight)	Grams of urine voided
17.....	79,380	44,524	374.2	93.389
18.....	79,380	3,101	396.6	54.632

usual standard methods. The results of the analyses are given in the following table:

TABLE II

PERCENTAGE COMPOSITION OF MILK, WATER, URINE AND DRIED FECES

			Urine		Feces	
	Milk	Water	No. 17	No. 18	No. 17	No. 18
Nitrogen.....	0.56	—	0.268	0.481	5.07	5.82
Ether ext.....	2.26	—	0.0	0.0	7.11	4.31
Sugar.....	4.20	—	0.0	0.0	nil	nil
Crude fiber.....	—	—	—	—	1.69	0.81
Ash.....	0.709	—	0.3165	0.5886	25.88	28.04
Calcium.....	0.108	0.0151	nil	nil	9.17	8.02
Phosphorus.....	0.0908	nil	0.0329	0.612	0.472	0.464

A small amount of crude fibre appeared in the feces due in part, no doubt, to the fact that the calves were working constantly to get bits of straw or shavings into their mouths and occasionally they were successful in doing so.

Table III shows the balance of nutrients during the seven-day metabolism trial.

The relatively low retention of nitrogen, 39.4 per cent for No. 17 and 35.7 per cent for No. 18, is no doubt due to the use of protein for energy. The twenty-five pounds of milk, which each calf received per day, made up of a mixture of two-fifths whole milk and three-fifths centrifuged milk, furnished slightly more protein than was required for normal growth, but did not furnish sufficient net energy, so the energy rather than the protein was the limiting factor in determining growth.

The results show that the two calves retained on the average 8.0 grams of calcium and 5.3 grams of phosphorus per day.

TABLE III

BALANCE OF NUTRIENTS DURING THE SEVEN-DAY METABOLISM TRIAL

	Calf No. 17				Calf No. 18			
	Grams fed	Grams eliminated	Balance	Per cent retained	Grams fed	Grams eliminated	Balance	Per cent retained
Nitrogen....	444.5	269.3	+ 175.2	39.4	444.5	285.9	+ 158.6	35.7
Ether extract	1794.0	26.6	+1767.4	98.3	1794.0	17.1	+1776.9	99.0
Ash.....	562.8	392.4	+ 170.4	30.3	562.8	432.8	+ 130.0	23.1
Calcium.....	92.4	34.4	+ 58.1	62.8	86.2	31.8	+ 54.4	63.1
Phosphorus..	72.1	32.5	+ 39.6	54.9	72.1	35.2	+ 36.9	51.2
Sugar.....	3334.0	0.0	+3334.0	100.00	3334.0	0.0	+3334.0	100.0

TABLE IV

COEFFICIENT OF DIGESTIBILITY OF MILK WHEN USED AS AN EXCLUSIVE DIET FOR CALVES

Nutrient	No. 17			No. 18		
	Grams in feed	Grams in feces	Coefficient of digestibility per cent	Grams in feed	Grams in feces	Coefficient of digestibility per cent
Nitrogen.....	444.5	19.0	95.7	444.5	23.1	94.8
Ether extract.....	1794.0	26.6	98.5	1794.0	17.1	99.0
Sugar.....	3334.0	nil	100.0	3334.0	nil	100.0

Henry and Morrison (3) give the following as the average coefficient of digestibility of milk by calves: Crude protein, 94 per cent; nitrogen-free extract, 98 per cent; fat, 97 per cent. A comparison of these average results with the results in Table IV shows clearly that the ability of the calves to digest food was not impaired by restricting them to a milk diet for a period of about eight months. This is of interest in view of the fact that it is generally believed that roughage is necessary in the diet of calves. No doubt roughage plays a part in the development of the muscles of the paunch and intestines. These results, however, indicate that this development is not necessary for the secretion of the digestive juices.

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2. Armsby, H. P., The Nutrition of Farm Animals, New York, 1917, 713.
3. Henry, W. A., and Morrison, F. B., Feeds and Feeding. Madison, Wisc., 18th Ed., 1923, 727.

THE EFFECT OF MINERAL OIL ADMINISTRATION UPON THE NUTRITIONAL ECONOMY OF FAT-SOLUBLE VITAMINS

I. STUDIES WITH THE VITAMIN A OF BUTTER FAT

By

RICHARD W. JACKSON

(*From the Laboratory of Physiological Chemistry, Yale University, New Haven.*)

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IN VIEW of the fact that many of the natural edible fats are soluble in medicinal mineral oils and conversely that the mineral oils are soluble in the fats, one may well be puzzled as to the probable alimentary fate of a third substance mutually soluble in the first two when all three are administered together orally to an animal. It is rather generally accepted that hydrocarbons such as mineral oils, contrary to the behavior of the natural dietary fat, are not absorbed unless perhaps in very small and ordinarily rather inappreciable amounts (cf. Bradley and Gasser, 1911; Bloor, 1913; Mellanby, 1927, and Channon and Collinson, 1929). The question, then, arises: How is this third substance, *e.g.*, one of the vitamins contained in fat, ultimately distributed in the alimentary tract between mineral oil, which finally passes unabsorbed out of the gut, and the fat which for the most part undergoes digestion and eventual absorption through the intestinal wall into the body? It seems logical that mineral oil might dissolve and retain significant though minute quantities of important accessory substances such as the fat-soluble vitamins. Should this occur, the body might be deprived of a portion of its indispensable materials, perhaps even suffering encroachment upon the absolute minimum necessary for health and well-being. Similarly, although surely much more improbably, mineral oil might cause such depletion by chemically inactivating the necessary substance, *e.g.*, vitamin A. These conceptions lead to the recognition of a possible limitation in the very large consumption of mineral oil as a therapeutic agent in the treatment of constipation, a limitation quite aside from the mineral oil characteristics of being apparently pharmacologically innocuous and non-habit forming. As has been pointed out in the editorial columns of the Journal of the American Medical Association (1927), the problem, on account of its general importance, deserves a thorough investigation.

A few brief reports concerning the effect of mineral oil administration to animals under observation with respect to their conservation of vitamins A and D have already been published. Burrows and Farr (1927) found death to occur with little delay among rats ingesting diets containing butter fat at a 17 per cent level, and mineral oil in the rather excessive proportions of 8 to 25 per cent. Study of their data leads one to the conclusion that the experimental animals died from factors associated, indeed, with the very large intake of mineral oil but *not* through the secondary production of a vitamin A deficiency. Dutcher, Ely, and Honeywell (1927) observed that mineral oil mixed with butter fat may prevent a considerable portion of the vitamin A in this butter fat from being available to rats previously exhausted of their vitamin A reserve. Failure of growth resulted whether daily doses of 67, 133, or 200 mgm. of butter fat—diluted to a total of three and one-half times the original weight with mineral oil—were given. Xerophthalmia developed in those animals receiving the smallest allotments of the butter fat-mineral oil combination. However, 67 mgm. of butter fat alone were sufficient to permit growth and to prevent the onset of eye lesions. More recently Ely, Honeywell, and Dutcher (1929) reported confirmation of their former findings. Again, Moore (1929) investigating carotene as a source of vitamin A noted that: "Poor growth responses . . . were given in some cases, and notably in those in which the carotene was administered in medicinal paraffin." And Hawk, Levine, Stucky, and Oser (1929) state: "Data from curative experiments on rats depleted of their bodily reserves of vitamin A, indicate that mineral oil lowers the utilization of this vitamin in cod liver oil." These citations would appear, perhaps, to constitute a considerable indictment of mineral oil therapy. Yet it must be borne in mind that to mix mineral oil at once and intimately with the entire daily vitamin A ingestion is contrary to ordinary human practice.

Moness and Christiansen (1929), on the other hand, decided that liquid petrolatum (Squibb) is "unobjectionable" and "even suitable as a vehicle for vitamin administration." Furthermore, Marcus (1929), possibly uncritical with respect to our present theme, proposed the use of mineral oil as a protective solvent for vitamin A; and Collison, Hume, Smedley-Maclean, and Smith (1929) with some success essayed its use as a carrier likewise for vitamin A, although the latter authors remark that "the effect seemed rather better with the hardened cotton-seed oil." They refer also to a finding of Hume and Smith (unpublished) that a certain fraction of an extract of spinach "is an adequate source of vitamin A, when dis-

solved in liquid paraffin." Finally, the experiments of MacDonald, Andes, and Briggs (1930), with mineral oil as an extraction agent in connection with milk, have a bearing on the point in question. On the basis of colormetric tests, they conclude: "The results show no decrease in vitamin A content because of mineral oil treatment for the removal of the onion flavor and odor from milk."

In our own investigation dealing with the general question of the effect of mineral oil on the nutritional economy of the fat-soluble vitamins, we elected first to repeat the experiments of Dutcher *et al.*, in which the butter fat was fed at the 67 mgm. daily dosage. In Chart 1 are presented protocols of our results which substantiate the findings of these authors in that 167 mgm. of mineral oil (in our case, two well-known medicinal brands) when mixed with an adequate but marginal 67 mgm. intake of butter fat, considerably decrease the availability of the vitamin A.

We have been interested chiefly, however, in determining the influence of mineral oil administered to experimental animals under conditions approximately those customary in the human usage of this therapeutic agent. Particularly have we aimed to study the effect exerted by mineral oil when introduced into the alimentary tract not incorporated with the butter fat but at certain time intervals reasonably distant from the dispensing of the vitamin A-bearing substance.

Plan of Experiments

Animals. Since most of the available information concerning vitamin A has been obtained through use of the rat, the present study was carried out with this species. Not only is vitamin A required by both rat and man, but its deficiency leads to many similar symptoms in the two forms. Furthermore, the action of mineral oil obviously must be localized in the alimentary tract and here again we find no marked difference in anatomy and functioning. Both the rat and man are omnivorous. Thus the rat would appear to provide fairly comparable alimentary conditions for the mixing and intermingling of the food mass, the vitamin in question and the mineral oil. Both male and female rats were used in the first experiments; subsequently only males were employed.

Diet. A conventional diet described in Table I was formulated for use in depleting the fats of

Table I

Composition of the Basal Diet (Without Added Vitamin A)

	Total Weight	Total Calories
	per cent	per cent
Casein, extracted	18	16
Starch, cooked	63	55
Salts, Osborne and Mendel (1919)	4	—
Hydrogenated vegetable oil*	15	29
	100	100

* Crisco.

Vitamins B, G, and D were supplied daily apart from the rest of the ration in the form of 400 mgm. of dried yeast (Northwestern Yeast Co.) irradiated according to Hess (1927).

vitamin A and subsequently in the testing of the butter fat given with and without mineral oil. Large quantities (25 kg.) of casein were prepared at one time. Each batch (1000 gm.) of technical casein was extracted successively with 3000 cc., 2500 cc., and 2500 cc. of boiling 95 per cent alcohol (cf. Osborne and Mendel, 1921) for one, one and one-half, and two hours respectively. The mother liquor of the third extraction of one batch was used for the first extraction of the next. Each extraction was immediately followed by filtration with suction and by a washing with 500 cc. of alcohol. The material was then extracted for one hour in 2500 cc. of boiling ether, filtered, then dried in air and finally heated for 48 hours in an oven at 100° C. The starch was prepared by pouring a cold suspension of cornstarch[1] into boiling water with vigorous stirring for a few minutes in order to cause complete gelatination. The resulting product was then dried and ground. Fat, an important constituent of human food, was included in the diet for two reasons: first, because it has been claimed by various writers that some fats under certain conditions exert a destructive action upon one or another of the fat-soluble vitamins, and secondly, because it appeared probable that fat in the intestine would ordinarily compete with the mineral oil for the vitamin A. In order to have such destructive action and such competition operating in a normal manner, whatever that might be, we endeavored to duplicate the fat content of a liberal American dietary[2]—about 30 per cent of the total calories according to Murlin and Hildebrandt (1919). Rats ingesting this basal diet developed eye symptoms in the minimum time of 18 days and in the average time of 24 days, with growth ceasing in the majority of animals shortly after the eyes became involved.

The preparation, dosage, and administration of the mineral oil and butter fat. Several pounds of creamery butter[3] were melted and allowed to separate in the usual manner whereupon the fat was filtered through a hot-water funnel. The material, placed in 300 cc. bottles, was stored in a container charged with carbon dioxide gas and kept in the refrigerator. About once a month a convenient amount was removed and divided into portions sufficient for a week. At the beginning of each week the old supply was discarded for a new one, so that no daily sampling was done after the butter fat had been melted more than seven or eight times. All lots of material in current use were kept in the dark and at about 5° C. In dispensing the butter fat, the practice was to warm it to a temperature of 50° C. and then to draw it into a special pipette designed and calibrated to deliver drops of 25 ± 0.5 mgm. Delivery was made onto inverted, clean and dry crucible lids from which the ring handles had been removed. These fat portions were then allowed to chill in the ice box until time for distribution among the experimental animals. Assay, involving the effect on growth and eye condition of the rat, revealed that the butter fat preparation just described was inadequate when supplied in 50 mgm. daily dosage, more nearly adequate at a 75 mgm. level and approximately adequate at a 100 mgm. level.

All the mineral oil-vitamin A experiments so far reported in any detail

[1] Duryeas' brand.

[2] The data for computation of this average were secured at various United States Army Training Camps during the World War.

[3] "Sunlight" brand.

have dealt with the rather indefinite substance "mineral oil." We employed two commonly used standard brands of material, representative of the paraffin and naphthene types of petroleum. These, we shall henceforth call Mineral Oils No. 1 and No. 2.

Choice of the amount of oil to be given to a rat was governed by two considerations: 1.—comparison with the human therapeutic dose and 2.—the quantity necessary to produce a detectable effect on both the character of the feces and the motility of the gut. Inasmuch as mineral oil presumably escapes absorption and, therefore, is confined in its action to the alimentary tract, we could find no compelling reason for comparing human and rat dosages, as some have done, on the basis of body weight. It appeared far more reasonable to make the comparison on the basis of the gut capacity or food content calculated from the caloric intake. The approximate rat:man weight ratio is 250:75,000. The approximate caloric ratio is 50:3000. Fantus (1920) gives the adult human dose as 15 to 90 cc. If we choose 30 cc. (two tablespoonfuls) as a reasonable amount to be taken continually (daily) over a long period of time, the weight comparison indicates 30/300 or 0.1 cc. and the caloric comparison, 30/60 or 0.5 cc. as the therapeutic dose for a rat.

It was found that, although a daily intake of 0.2 cc. of mineral oil did not noticeably alter the external appearance of the rat feces, 0.5 cc. of either mineral oil produced a pronounced alteration in their character. They were softer and larger, and often were coated with a thin film of oil, and analysis revealed a greater water content. Furthermore, the 0.5 cc. intake of mineral oil was shown to cause a rather moderate but nevertheless definite acceleration of intestinal motility. Data to support this last statement will be presented in a separate communication.

On the grounds of the foregoing evidence, 0.5 cc. was adopted as the standard rat dose of mineral oil in these experiments. This was given every day except Sunday in three separate 0.17 cc. doses within the following hours: 8 to 9 a.m., 2 to 3 p.m. and 5 to 6 p.m. By means of a 1 cc. insulin syringe, the oil was delivered through a small glass tube well to the rear of the pharynx. The soiled tube was replaced always with a clean one before proceeding with the next animal. It was found that a rat could handle as much as the 0.17 cc. administered in this way without any overflow about the mouth that might result in subsequent loss. The procedure was justified by repeated observation of the animals' habits. Aside from occasionally displaying certain refractory reactions to the mineral oil, the rats took the material well and even, in some instances, soon became accus-

tomed to coöperate in its reception. Again, conclusive evidence that the rat actually ingested the oil given was secured by demonstrating that it could be practically quantitatively recovered from the feces as ether-soluble material.

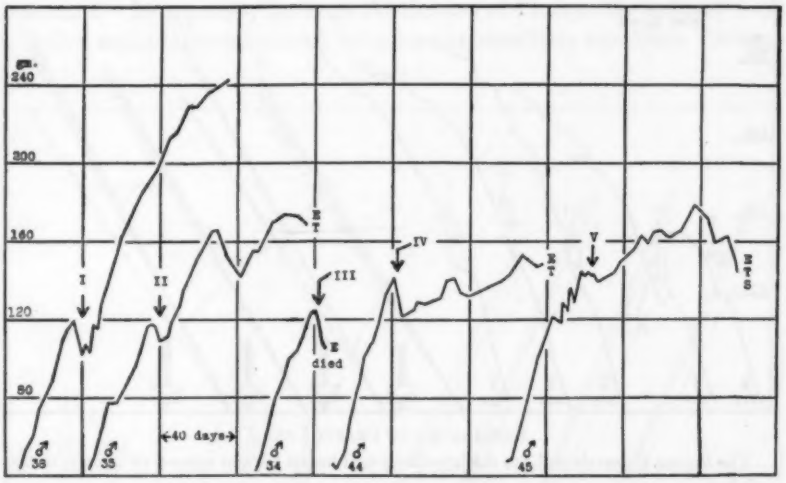
The butter fat was dispensed every day of the week during the hour of 11 to 12 a.m. and was almost invariably immediately and completely consumed. Thus, a stream of mineral oil was kept flowing through the rat's alimentary tract with obvious opportunity for the oil to make contact with other material ingested between the doses of the oil. Yet three hours always elapsed between the butter fat and mineral oil administrations. This program represented what appeared to be a rigorous but fair trial of the action of mineral oil on vitamin A in the gut under conditions similar to those attending the use of mineral oil by man.

Conduct of experiments; indices of vitamin A deficiency. Young albino rats at about the weaning age of 21 days and always within the weight range of 40 to 50 gms. were removed from the mothers and from the stock diet.[4] They were placed in individual cages equipped with large-meshed wire false bottoms, given the basal vitamin-deficient diet, and allowed to grow until definite avitaminosis was evidenced in either eye lesions or lack of growth or both. In case of occasional considerable delay in the development of a positive sign in one of the two indices, the other was accepted as an indication to proceed with the experiment. This plan avoided carrying the deficiency to the point of quick general debacle. Like others, we have also recognized the possibility of depleting animals to the degree that there occurs irreparable damage whose manifestation may be subtle and long delayed. These dangers we have tried to avert by using a standard depletion interval arranged to be slightly less than the minimum time required for unmistakable eye symptoms to occur. We have attempted also to use the depletion time of the animal first becoming deficient as a signal to begin the experiments with the remaining members of the litter. However, it seemed necessary to abandon these procedures in favor of the scheme of definitely depleting each rat. The vaginal smear method, though claimed to provide a sensitive index for the measurement of vitamin A exhaustion in the female rats, was of very little use because in many instances the vagina did not open until several days after other deficiency symptoms had been well established (*cf.* Coward, 1929). Nevertheless, there appeared to

[4] The rats whose growth curves are depicted in Charts 1, 2, and 3 were reared on Sherman's Stock Diet B somewhat modified; the remainder of the animals (See Charts IV and V) on a calf meal diet (Maynard, 1930). Both of these diets were supplemented at particular times by separate administration of cod liver oil, fresh lettuce, dried yeast, etc.

us several possible reasons why the experimental animals to be used in the comparisons that have been outlined here should be in even a more certain state of vitamin balance than that required of animals depleted simply for the purpose of demonstrating the curative power of some natural vitamin A-containing substance. To offset the rapid development of irreversible changes, therefore, each rat after being satisfactorily depleted was given

CHART 1.



EXPLANATION OF CHART 1

The Roman numerals indicate the introduction of butter fat and mineral oil in daily doses as follows:

I. 67 mgm. of butter fat.
II. 33 mgm. of butter fat.
III. No butter fat.
IV. 67 mgm. of butter fat mixed with 167 mgm. of Mineral Oil No. 1.
V. 67 mgm. of butter fat mixed with 167 mgm. of Mineral Oil No. 2.

Letters immediately adjacent to the end of a curve are used to record necropsy findings:

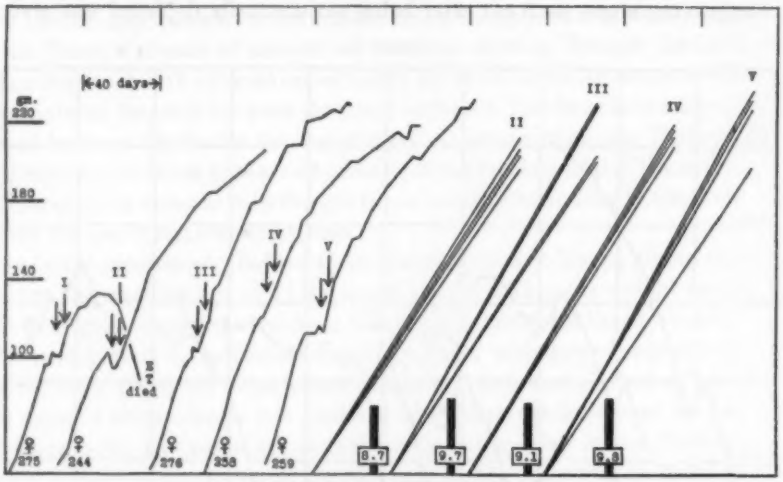
E. Definite ophthalmia of varying severity.
T. Abscess formation in the tongue.
S. Abscess formation in the submaxillary glands.

The growth curves presented in this chart are representative of the results secured in carrying out a repetition of the experiments of Dutcher, Ely, and Honeywell (1927). The basal diet used by these authors both for depleting the rats of vitamin A and for the subsequent test periods contained casein, dextrin and salts but no fat. Every described detail of their experimental plan was accurately duplicated, with the exception that in our experiments, each individual animal was kept upon one régime for the longer term of 75 days. The butter fat which we employed was from a Lot No. 1 which was a different lot than that referred to later in this paper. Fresh mixtures of the butter fat and mineral oils were prepared every twelve days and were always kept chilled and in the dark.

the full experimental dose of vitamin A for four days before it was transferred to one of the experimental régimes.

The animals were divided among the various experimental groups with attention to an equitable distribution as to sex, weight, age, eye condition,

CHART 2.



EXPLANATION OF CHARTS 2 AND 3

The Roman numerals indicate the introduction of butter fat and mineral oil in daily dosages as follows:

I. No butter fat.
II. 75 mgm. of butter fat.
III. 150 mgm. of butter fat.
IV. 150 mgm. of butter fat and 0.5 cc. of Mineral Oil No. 1 *separately* (see text).
V. 150 mgm. of butter fat and 0.5 cc. of Mineral Oil No. 2 *separately*.

Letters adjacent to the ends of the protocol curves represent necropsy findings:

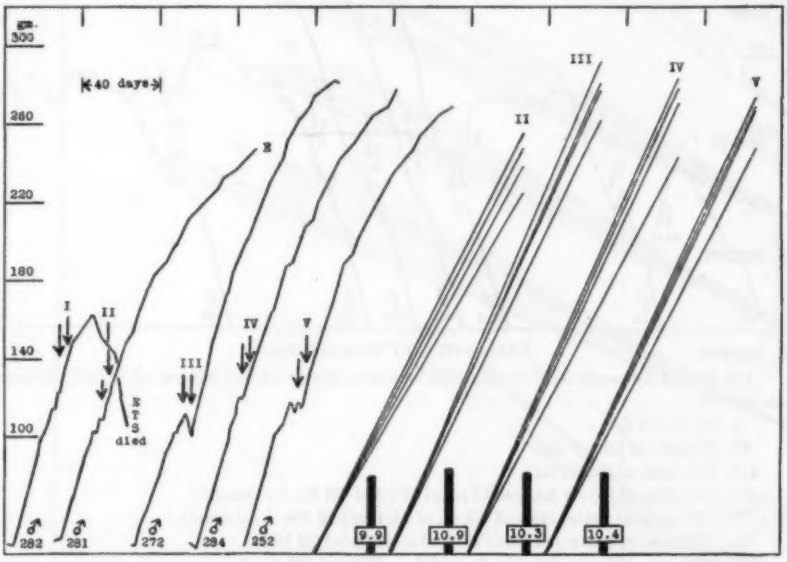
E. Definite ophthalmia of varying severity.
T. Abscess formation in the tongue.
S. Abscess formation in the submaxillary glands.

GENERAL EXPLANATION OF CHARTS 2, 3, 4, AND 5

The growth curves depicted in these charts are of two kinds: 1. representative curves plotted in detail without smoothing to serve as protocols and 2. straight line graphs to facilitate group comparison. The latter were prepared by drawing lines from an arbitrary common origin representing 40 gm. to points representing the final weights (in gm.) attained. The corresponding abscissae represent the average depletion time, 26 or 28 days, plus the recuperation interval, 4 days (see text), plus the experimental period, 75 days; that is, 105 days in Chart 5 and 107 days in Charts 2 and 3. The black columns show averages of group food consumptions in grams. Of the two arrows shown in the protocol curves, the first records the time when the animal was adjudged to be definitely depleted of vitamin A: the second, the end of the four-day readjustment period. The butter fat used in all experiments described in these Charts is from a Lot No. 2, a different lot than that referred to in Chart 1.

etc. The groups may be labeled and briefly described as follows: the negative controls receiving no butter fat; the controls receiving one-half of the full amount of butter fat; the controls receiving the full amount; and finally the test animals receiving either of the mineral oils along with the full amount of the butter fat. Vitamin A deficiency was gauged as follows: 1.—by growth, the animals being weighed twice a week; 2.—by eye condition, the eyes being examined carefully once a week; 3.—by vaginal smears; 4.—by necropsy findings. At the end of 75 days the animals were all killed and a thorough search for gross abnormalities was made. Particu-

CHART 3.



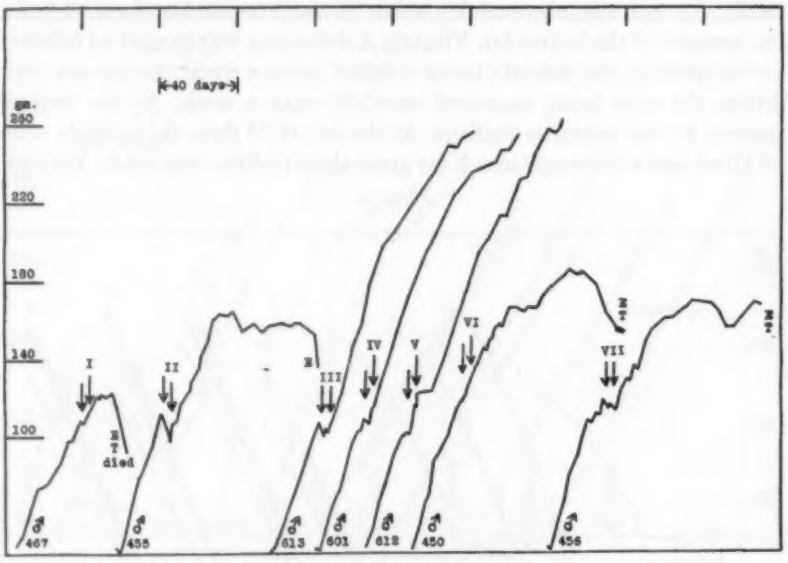
lar attention was paid to the occurrence of abscesses in the glands in the base of the tongue since such pathological conditions have been found in this laboratory both by Tyson and Smith (1929) and by ourselves to appear quite uniformly among animals reaching a certain degree of severe vitamin A exhaustion (cf. Sherman and Munsell, 1925). Two large series of experiments were carried out, one with 150 mgm. and a second with 100 mgm. of butter fat as the amount fed.

DISCUSSION OF RESULTS

Inasmuch as some of our first experiments designed to furnish the test animals barely an adequate amount of butter fat were difficult of interpre-

tation, because all the growth curves, control and otherwise, fluctuated considerably, we decided in the present studies first to ascertain the effect

CHART 4.



EXPLANATION OF CHARTS 4 AND 5

The Roman numerals indicate the introduction of butter fat and mineral oil in daily dosages as follows:

I. No butter fat.
II. 50 mgm. of butter fat.
III. 100 mgm. of butter fat.
IV. 100 mgm. of butter fat and 0.5 cc. of Mineral Oil No. 1 *separately*.
V. 100 mgm. of butter fat and 0.5 cc. of Mineral Oil No. 2 *separately*.
VI. 100 mgm. of butter fat *mixed with* 0.5 cc. Mineral Oil No. 1.
VII. 100 mgm. of butter fat *mixed with* 0.5 cc. Mineral Oil No. 2.

Letters adjacent to the ends of the curves represent necropsy findings:

E. Definite ophthalmia of varying severity.
T. Abscess formation in the tongue.
S. Abscess formation in the submaxillary glands.
K. Abscess formation in the kidney.
B. Bladder stones.

ADDITIONAL EXPLANATION OF CHART 5

(*See opposite page.*)

Since all graphs are arbitrarily drawn as though every rat survived the 75-day experimental period, it should be noted that all rats of Group I and one rat each of Groups II, VI and VII died within the 75 day period. Numbers used as coefficients in connection with the necropsy findings record the total separate cases in which a finding was made. The group average final weights are represented by a horizontal line marked with a circle.

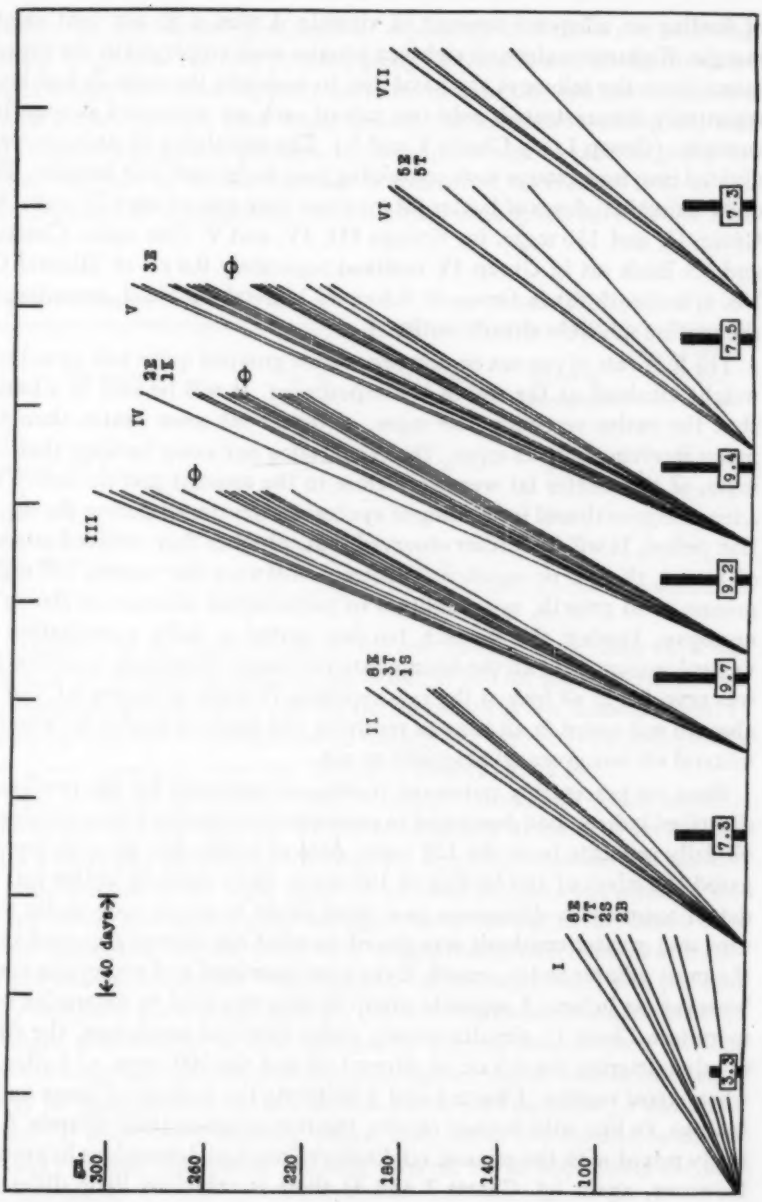


CHART 5.
gm.
300
280
220
180
140
100
K-40 days-K
I
6E
7T
2S
2S
3.3
II
6K
3T
1S
7.3
III
Φ
9.7
IV
2E
1K
Φ
9.2
V
3E
Φ
9.4
VI
5E
5T
7.5
VII
7.3

of feeding an adequate amount of vitamin A plus a 50 per cent safety margin. Eighteen males and eighteen females were employed in the experiment. Since the failure of the basal diet to maintain the animals had been repeatedly demonstrated, only two rats of each sex were used as negative controls. (Group I, see Charts 2 and 3.) The remaining 32 animals were divided into four groups each containing four males and four females. The daily individual doses of butter fat for these four groups were 75 mgm. for Group II, and 150 mgm. for Groups III, IV, and V. (See again Charts 2 and 3.) Each rat in Group IV received separately 0.5 cc. of Mineral Oil No. 1, and each rat in Group V, 0.5 cc. of Mineral Oil No. 2, according to the routine schedule already outlined.

The four rats of one sex on any one régime grouped quite well as to body weight attained at the end of the experiment. It will be seen in Chart 2 that the males receiving 150 mgm. of butter fat grew better than the males receiving only 75 mgm., thus confirming our assay findings that 100 mgm. of this butter fat were very close to the amount just necessary for adequate growth and for healing of eye lesions developed during the depletion period. It will be further observed that, whether they received mineral oil or not, there is no significant difference between the various 150 mgm. groups as to growth, eye condition or pathological changes as shown at necropsy. During the seventh ten-day period a daily examination of vaginal smears from all the female rats was made. Persistent cornification was revealed in all four of the rats receiving 75 mgm. of butter fat, but its absence was noted in all females receiving 150 mgm. of butter fat whether mineral oil was given additionally or not.

Since no perceptibly untoward results, as measured by the previously described indices, had developed in consequence of giving 0.5 cc. of mineral oil daily separate from the 150 mgm. dose of butter fat, we next investigated the effect of the feeding of 100 mgm. daily doses of butter fat. To detect appreciable differences now more likely to occur, only males were used and greater emphasis was placed on what has always appeared to be the most reliable index, *growth*. Eyes were examined and necropsies made, however, as before. A separate group of rats was used to determine once more (see Chart 1), simultaneously under identical conditions, the effect of administering the 0.5 cc. of mineral oil and the 100 mgm. of butter fat when *mixed together*. Charts 4 and 5 illustrate the findings of these undertakings. In line with former results, the rats securing their vitamin A already mixed with the mineral oil displayed marked depressions in growth. However, again (*cf.* Charts 2 and 3) there is exhibited little difference

between the rats receiving mineral oil separately and those not receiving it at all. In general the rats in these groups have attained about the same weight levels. Nevertheless, some instances of unhealed eye lesions and one case of kidney abscess were found among the animals receiving oil, and what is more significant, perhaps, the weight average of each oil group including fourteen to sixteen animals is somewhat lower than that of the control group. It follows from the statistical data given in Table II that

TABLE II

MEANS AND STANDARD DEVIATIONS (SIGMA) OF MEAN DIFFERENCES OF FINAL BODY WEIGHTS

Rat group	No. of animals	Final weight mean	Sigma of distribution	Sigma of the difference in means
		gm.	gm.	gm.
Mineral oil No. 1	14	247.0	19.0	
				8.8
Control	14	267.0	24.0	
				8.8
Mineral oil No. 2	16	253.0	24.3	

the chances that the mean weight of the controls is greater than the mean weight of the mineral oil animals are 99 out of 100 for the Mineral Oil No. 1 comparison, and 94 out of 100 for the Mineral Oil No. 2 comparison. It is fairly certain, then, that mineral oil slightly but definitely retards growth when given separately from the 100 mgm. dose of butter fat.

These observations indicate that, although mineral oil (both brands), when mixed directly with an otherwise adequate amount of butter fat, exerts a markedly harmful effect upon the vitamin A economy of the rat, the same amount of the same oil, given in doses separate but seriatim, beginning before and ending after the ingestion of the butter fat, causes only a slight depression of growth and only a questionable increase in ophthalmia and abscess formation. They indicate further that when a 50 per cent safety margin of the vitamin is present, no detectably adverse action occurs as judged additionally in this instance by vaginal smears. It is obvious from the foregoing experiments that although a vitamin A deficiency such as may be brought about *by mixing mineral oil with the butter fat* should be avoided, it does not necessarily follow that under the conditions of the *customary human therapeutic practice*, mineral oil is responsible for any such deficiency.

SUMMARY

1. Mineral oil causes a considerable loss of vitamin A to the animal organism, if the mineral oil is *mixed with* the vitamin A (in the form of butter fat) prior to ingestion.

2. The administration of the mineral oil *separately* from the butter fat results in only a very slight diversion of vitamin A.

3. Moderate increase of the butter fat intake appears to protect the animal from any vitamin A deficiency when the mineral oil is given separately.

In conclusion, the writer wishes to acknowledge the helpful suggestions made by various members of the Department, especially those made by Professor Lafayette B. Mendel.

ADDENDUM

Several months after this paper was submitted for publication, there appeared a contribution by Rowntree (1931) on the "The Effect of the Use of Mineral Oil Upon the Absorption of Vitamin A." The results obtained in many respects comparable to those reported here will be commented upon in a later communication.

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VITAMIN A DEFICIENCY IN THE ALBINO MOUSE*

By

J. M. WOLFE AND H. P. SALTER, JR.

(From The Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee.)

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THAT the epithelium of the various structures of the body undergoes extensive changes when there is a deficiency of vitamin A in the diet, has been shown in the case of man by Wilson and DuBois (14), and for the rat and the guinea pig by the work of Wolbach and Howe (15, 16). In man and the rat, the normal epithelium of the respiratory tract, the alimentary tract, the genito-urinary tract, and the eye and paraocular glands is desquamated and is replaced by keratinized epithelium. The guinea pig shows similar changes, although the eye and paraocular glands are usually not affected.

Wolbach and Howe state that the keratinization can take place in the absence of infection and that, if infection does occur, it is secondary to the changes in the epithelium which, they think, are due directly to the lack of vitamin A. On the other hand, Tyson and Smith (13) have pointed out that infection is always present, even in the earliest stages of the deficiency and, in the later stages, always dominates the picture.

Nelson and Lamb (9) fed rabbits on a synthetic diet to which alfalfa, extracted with alcohol, had been added. These animals developed ophthalmia which was relieved by adding butter fat to the diet. Steenbock, Nelson, and Hart (12), in their studies on calcium assimilation, found that dogs developed ophthalmia on the diet used. They were able to cure this ophthalmia by adding vitamin A to the diet. Beach (1) has reported that fowls fed on a diet deficient in vitamin A suffer from ophthalmia, nasal discharge, lesions of the mucosa of the mouth, pharynx, and esophagus; and, in the later stages, become weak and emaciated.

Beard (2), in his studies on the nutrition of the white mouse, was unable to produce the typical symptoms of xerophthalmia in white mice fed on a diet deficient in vitamin A, even though the other symptoms of A deficiency were present, *i.e.*, arrest and decline of growth, and roughened fur. Spinka (11), while studying the effect of ultra-violet rays on the accessory food substances, found that his mice developed a typical xerophthalmia

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and died in about 50 days. Fujimaki (4), in studying the formation of urinary and bile duct calculi in animals fed on various experimental diets, found that mice fed on a diet deficient in vitamin A, developed xerophthalmia and died in about 25 days.

Recently Pomerene and Beard (10) have made a further study of this question and, contrary to Beard's first results, they have been able to produce xerophthalmia in the mouse in 50 to 60 days. In these experiments they were unable to produce xerophthalmia on any diet that contained hydrogenated vegetable fat[1] unless the fat was aerated before use. From these results they came to the conclusion that the fat used contains enough A to protect mice from the typical eye symptoms, although it does not contain sufficient A to promote good growth. They attribute their failure to produce xerophthalmia in earlier experiments to the fact that their diet contained 24 per cent hydrogenated vegetable fat[1]. It is well known, from the results of many investigators, that the presence of this fat in a diet otherwise deficient in vitamin A will not prevent xerophthalmia in rats. Thus Pomerene and Beard conclude that the requirements of rats and mice for the anti-xerophthalmic factor are different, rats requiring vitamin A at a higher level than do mice.

We have made a study of A-deficiency in white mice to determine whether or not they undergo the extensive changes of the epithelium throughout the body which have been described by Wolbach and Howe for the rat and guinea pig. In this study we have used 50 mice, both males and females being included in the series. They were placed on a diet when they had reached a weight of 9 to 12 grams. In all cases litter mate brothers or sisters were used as controls. All except the first series, which consisted of twelve mice, were kept in separate wire cages with a mesh bottom. Those of the first series were kept in two groups of six. The following experimental diet was used:

	Per cent
Casein (purified)	31
Dextrin	35
Hydrogenated veg. fat[1]	22
Salts (Osborne and Mendel)	7
Yeast (Harris)	5

The control diet was the same, except that cod liver oil (Harris) was added at a 3 per cent level and the percentage of fat[1] lowered to 19.

The length of time required for the mice to develop xerophthalmia

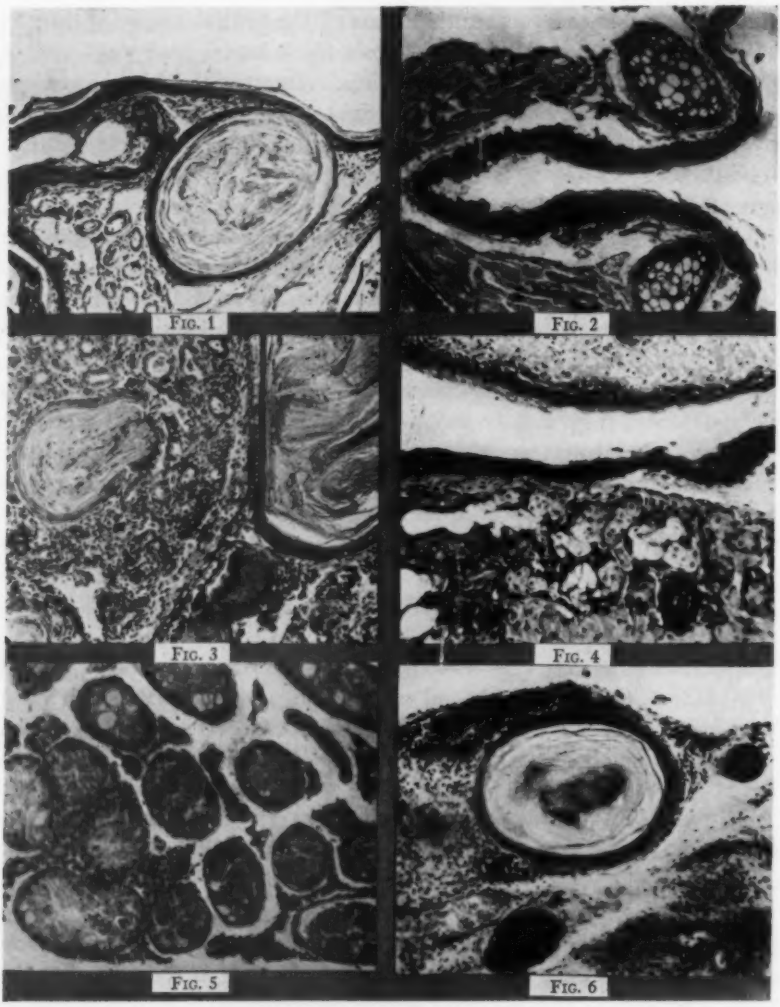
[1] Crisco.

varied greatly. In some cases they showed the typical eye symptoms in 35 days, but in a majority of the cases a much longer time was required (90 to 120 days). The experimental animals almost invariably showed eye symptoms before they began to lose weight.

The animals were killed at various stages of the deficiency in order to get as comprehensive a picture of the tissue changes as possible. Those animals which were allowed to develop the most advanced condition before being sacrificed had assumed a roughened coat and a humped posture which is the characteristic appearance of rats fed on an A-deficient diet. The eyes were encrusted with a granular appearing exudate which, in most cases, was not hemorrhagic as is usually found in rats. Placques of desquamated cells were found on the cornea. The animals became extremely weak and often were scarcely able to support themselves. At this stage of the deficiency, they usually weighed 13 to 15 grams. Almost invariably they exhibited respiratory distress. Diarrhea was also a common symptom in animals which were allowed to reach the later stages. The food intake in later stages of the disease was extremely low.

The gross postmortem changes were very marked. There was a complete disappearance of the body fat. The thymus was markedly atrophic in practically every animal. The salivary glands were usually about one-half normal in size. In many cases the lungs showed large hemorrhagic spots. The spleen was usually very dark in color and extremely small. The pelvis of the kidney was sometimes filled with a granular opaque mass, which was later found to be made up of desquamated cells. In one case a large renal calculus was found in the pelvis of the kidney. In a large number of animals the bladder wall was thickened and the bladder was filled with a mass similar to that found in the pelvis. The seminal vesicles were usually extremely distended and filled with similar masses. The testes were small soft, and usually very watery when cut.

The tissues from 16 animals were studied histologically. They gave a picture very similar to that described for the rat and guinea pig by Wolbach and Howe. They found that the various types of epithelium of the body organs were replaced by keratinized cells which usually began to grow in small clumps beneath the normal cells. These "foci" of cells grew by peripheral extension beneath the apparently normal epithelium. The original epithelium seemed able to survive for a considerable length of time, even though it was separated from its connective tissue support by the keratinized cells. Although we have observed these "foci" of cells in mouse tissues, the more common finding was an orderly layer of keratin-



DESCRIPTION OF PLATE

FIG. 1. Photomicrograph of a cross-section of the nares of mouse no. 86 which had been on experimental diet for 100 days. X 150.

FIG. 2. Photomicrograph of a cross-section of the trachea of mouse no. 84 which had been on experimental diet for 100 days. X 150.

FIG. 3. Photomicrograph of a cross-section of the submaxillary gland of mouse no. 84 which had been on the experimental diet for 100 days. X 150.

ized cells which seemed to begin at only one point and grow in all directions. We have never noticed several foci in any one section.

The first changes usually appeared in the respiratory tract (Figure 1), as described by Wolbach and Howe for the rat, but were closely followed by the symptoms of xerophthalmia. All stages of keratinization and desquamation were observed. In no case was there a purulent exudate in the nares. In some few cases the histological sections have shown some evidence of infection. In the nares, trachea (Figure 2), and bronchi there was extensive keratinization of the epithelium, accompanied by marked desquamation. In the lungs, bronchiectases were frequently found. They were probably caused by the occlusion of the bronchi by desquamated cells.

In the stomach and intestine no changes were evident. An extremely large amount of desquamation was observed in the esophagus. A similar desquamation, though in a much smaller degree, was observed in normal control mice. No constant changes were found in the liver. The submaxillary glands of our mice did not seem to be affected as early or to the same extent as Wolbach and Howe (15) found to be the case in the rat. We have not been able to demonstrate any change in the secreting epithelium except perhaps a slight atrophy. In the ducts, however, in the later stages of A-deficiency, we have observed all stages of the typical changes from a beginning of keratinization to a complete replacement of the normal epithelium by the keratinized type (Figure 3). In the latter case the ducts were completely filled with desquamated material. We have not observed any degenerative changes in the accessory salivary glands at the base of the tongue.

Extensive changes were noticed in the bladder and the pelvis of the kidney, the normal epithelium being replaced by keratinized epithelium. The bladder in many cases was completely filled with desquamated cells, while the pelvis of the kidney showed desquamation to a lesser degree. A leucocytic infiltration was often seen in the latter. In the female genital tract there were no noticeable changes. The ovaries, fallopian tubes, uterus and uterine glands appeared normal.

The testes showed various stages of degenerative changes. The first

FIG. 4. Photomicrograph of a cross-section of the cornea and the eye-lid of mouse no. 86 which had been on experimental diet for 100 days. X 150.

FIG. 5. Photomicrograph of a section of the testis of mouse no. 41 which had been on experimental diet for 119 days. X 150.

FIG. 6. Photomicrograph of a section of the prostate gland of mouse no. 48 which had been on the experimental diet for 79 days. X 150.

change noted was some desquamation of the germinal epithelium. As degeneration progressed, we found large amounts of a structureless material, which contained many clear spaces or vacuoles. It is probable that this structureless material was made up of masses of giant cells which had degenerated to such an extent that all cell structure was lost (Figure 5). That such giant cells are formed in the degenerative process, has been shown by Mason (7 and 8) in his work on rats, fed diets deficient in either vitamin A or E. In our experiments only the primary germ cells and the sustentacular cells were left in the tubules in the later stages. The tubules were shrunken and evidently had been surrounded by a watery fluid. At autopsy the testes were always soft and watery. No changes were noticed in the interstitial cells.

The prostate and seminal vesicles were, in most cases, greatly distended with desquamated masses (Figure 6). Histological examination showed that the original epithelium was completely replaced by keratinized epithelium. The lumina of the tubules were completely filled with sheets of desquamated cells, and were so distended that they appeared cyst-like. The connective tissue between the tubules was greatly increased. In many instances there was a down-growth of the keratinized epithelium into the subjacent connective tissue. These changes resemble those described by Wolbach and Howe for the rat and guinea pig. No changes were observed in the epithelium of the epididymis.

The adrenal and thyroid appeared normal. Although there was a complete or nearly complete atrophy of the thymus, histological examination of the persisting tissue revealed no constant changes.

The eyes and eyelids from fifteen mice have been studied histologically. Compared with other organs, the changes in the eyes were slight, although at autopsy a severe granular exudate was usually seen. The most outstanding feature was the keratinization and desquamation of the epithelium of the palpebral conjunctiva which, in some cases, was quite severe. The Meibomian glands showed various degrees of atrophy and vacuolization. The changes in the cornea were for the most part slight. In some cases there was a slight keratinization, accompanied by some desquamation. In a few instances a slight infiltration of leucocytes into the connective tissue of the cornea was noticed. Bowman's membrane was usually intact.

In one case the cornea had increased in thickness until it was 4 or 5 times as thick as is usually found. There was an extensive keratinization of the epithelium of both the cornea and the conjunctiva, accompanied by a large amount of desquamation. In the cornea there was a great increase in

the vascularity of the substantia propria, which was also very edematous. There was also an extensive infiltration of leucocytes. Bowman's membrane had disappeared and there was a down-growth of the keratinized cells into the connective tissue below. A considerable number of polymorphonuclear leucocytes were found in the anterior chamber of the eye. The animal referred to above was the only one in which an extreme eye condition was noted.

As shown by Wolbach and Howe for the rat, and we have found a similar condition in the mouse, the changes in the epithelium of the eye occur later than those of the respiratory tract, so that it would seem that the epithelium of the eye is less susceptible to the A-deficiency than that of some other organs of the body. A striking difference was found between the exudate exhibited by the eye of the mouse and that of the rat. In the mouse, in our experience, the exudate is usually not hemorrhagic, but is light in color and granular, while in the rat the exudate is very hemorrhagic.

Discussion

In the diet used in these experiments, the hydrogenated vegetable fat was the only source of vitamin E. It was supplied at a 22 per cent level. It has been shown by Kennedy and Palmer (6) that, when this fat is incorporated in the diet at a 15 per cent level, successful reproduction is secured. Evans and Burr (3) secured "cures" in sterile female rats by feeding it at a 22 per cent level. From these observations it seems evident that the diet we used contained a sufficient amount of vitamin E, and therefore that the severe testicular degeneration observed was due to the lack of vitamin A.

We have not found marked evidence of infection, even in the later stages of the deficiency. In many cases we have observed a complete replacement of the normal epithelium by the keratinized type, with no sign of infection present. In general, the histopathology found has been very similar to that described by Wolbach and Howe for the rat and guinea pig. The diet used was also deficient in vitamins C and D. However, it has been shown by the work of Goldblatt and Benischek (5) that the lack of these vitamins plays no part in the metaplastic changes, so it is evident that the keratinization observed is due directly to the lack of vitamin A in the diet. Since our experimental diet contained hydrogenated vegetable fat at practically the same level as used by Beard, we are at a loss to explain why our animals developed xerophthalmia, while his did not.

Summary and Conclusions

In both male and female mice, placed on an A-deficient diet at about 25 days of age, the normal epithelium of the various structures was replaced by keratinized epithelium. The mice showed evidence of xerophthalmia in 25 to 120 days. However, the severity of the eye symptoms was slight as compared with the changes undergone by other structures of the body. We have repeatedly observed metaplastic changes in the respiratory tract before any eye symptoms were present. The male mice suffered testicular degeneration similar to that described by Mason for rats, fed on both A- and E-deficient diets.

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THE VITAMIN VALUE OF COD LIVER MEAL

By

ARTHUR D. HOLMES, MADELEINE G. PIGOTT, AND DAVID F. MENARD

(From the Research Laboratories, The E. L. Patch Company, Boston.)

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COD LIVER MEAL is obtained as a by-product in the cod liver oil industry. In the manufacture of cod liver oil by direct stem process, a large portion of the oil and water-soluble proteins are removed from the liver tissues. The remaining liver tissue, after it is dried and ground, is sold as "cod liver meal." The physical appearance of cod liver meal varies greatly due primarily to the length of time which elapses between cooking the livers and the subsequent drying. When the liver residue is dried very promptly after the oil is extracted, a good quality of cod liver meal is obtained. On the other hand, when the liver residue is allowed to remain in a moist condition several months previous to drying, the resulting liver meal is a dark color and has a pronounced odor.

Considered from a nutritive standpoint, cod liver meal contains about 40 per cent of protein and nearly as much fat. In view of the relatively large amount of residual fat in cod liver meal, it has been frequently suggested that it could be used in poultry rations instead of cod liver oil as a source of vitamins. The present investigation was undertaken to secure data concerning the vitamin value of commerical cod liver meal.

Four lots of commercial cod liver meal were obtained on the open market. They were obtained from different sources and were apparently free from fish scraps. Three of the meals, 1, 2, and 4, were reported to be of Norwegian origin. The fourth meal, 3, was supposed to have been produced

TABLE I

RESULTS OF ANALYSES OF COD LIVER MEALS

Per Cent	Meal 1 (test 1)	Meal 2 (test 2)	Meal 3 (test 3)	Meal 4 (test 4)
Moisture	5.47	5.27	6.15	7.25
Ash	2.15	2.53	3.87	3.28
Protein	46.08	37.65	38.04	48.25
Fat	33.16	43.38	34.38	27.15
Carbohydrate	13.14	11.17	17.56	14.09
Calcium	0.09	0.02	0.21	0.18
Phosphorus	0.41	0.54	0.73	0.72

in Nova Scotia and Newfoundland. Samples of the meals were analyzed by the Official Methods of Agricultural Chemists (1) and were found to have the composition shown in Table I.

As will be noted from the results obtained, the chemical composition of the four meals varied widely. The greatest variation was in the protein and residual fat content. Lesser, but significant differences occurred in the calcium and phosphorus content of the meals.

Experimental Procedure

Rhode Island Red baby chicks, were housed in an all-metal type, electrically-heated brooder. The brooder was situated in the northern portion of the laboratory, where no direct sunlight was available. The chicks received an all-mash ration unsupplemented by grit or additional sources of lime. The composition of the all-mash ration was as follows:

Composition of Experimental Ration

	Per cent
Yellow corn meal, No. 2	30.0
Standard wheat bran	15.0
Wheat flour middlings	15.0
Ground oat groats	15.0
Dried buttermilk	8.0
Alfalfa leaf meal	5.0
Steamed bone meal	4.0
Fish meal (55% protein)	3.5
Meat scraps	3.5
Salt	1.0

The chicks were divided into eight groups (pens) of twenty each. Each group was allowed six square feet of space which was ample since four chicks were removed from each pen on the 21st, 35th, and 56th day of the test. The four experimental pens received the basal ration plus 2 per cent of cod liver meals 1, 2, 3, and 4 respectively, incorporated in the ration.

The tests of the cod liver meals were made at four different periods. In each instance, four pens (controls) were fed cod liver oil at four different levels, namely: 0.13, 0.25, 0.50, and 1.00 per cent of the ration. For the purpose of this discussion, the pen receiving cod liver oil which most nearly duplicated the results obtained with the cod liver meal under consideration has been selected as the "control" pen. Thus in test 1 the pen receiving 0.50 per cent cod liver oil, in test 2 the pen receiving 0.50 per cent cod liver oil, in test 3 the pen receiving 0.25 per cent cod liver oil, and in test 4 the pen receiving 0.13 per cent cod liver oil have been selected as "control" pens.

The chicks and food were weighed weekly as individual pens. The experi-

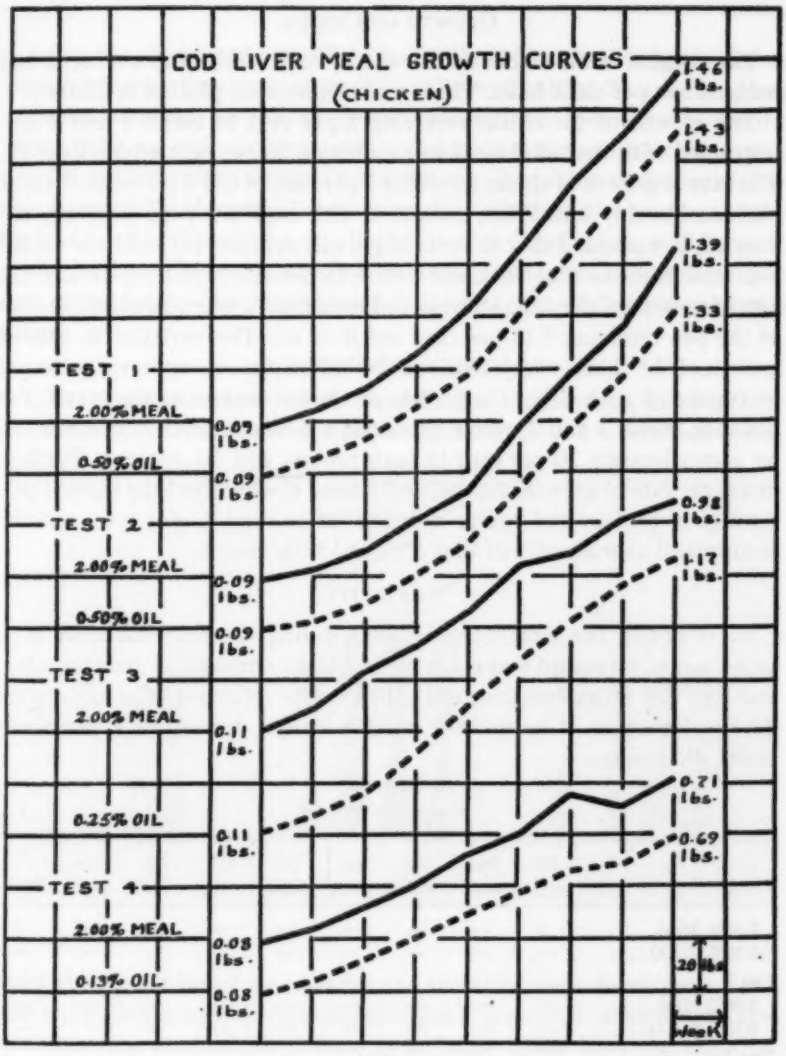

CHART 1.

ment was of eight weeks' duration. The value of the different cod liver meals as sources of vitamins has been judged by the general physical appearance of the birds, their rate of growth, mortality and their bone development.

Growth of Chicks

The weights of the chicks at the end of each week were averaged and reduced to a per chick basis. These results have been plotted in Chart 1.

The growth of the chicks receiving 2 per cent of meals 1 and 2 corresponded with that of the chicks receiving 0.50 per cent of cod liver oil. The rate of growth of chicks receiving 2 per cent of cod liver meals 3 and 4 was considerably less. When compared with duplicate pens receiving cod liver oil, it was found that the rate of growth obtained for cod liver meal 3 was equivalent to that obtained with 0.25 per cent cod liver oil and the rate of growth of the pen receiving cod liver meal 4 was equivalent to that of the pen receiving 0.13 per cent cod liver oil. The variation in rate of growth of the chicks which received the different meals apparently cannot be correlated with either the protein or the fat content of the meals. For instance, meals 1 and 2, which produced a similar growth curve, differed by approximately 10 per cent in both protein and fat content. Furthermore, the rate of growth obtained with meal 4, which had the highest percentage of protein and nearly as much fat as meal 1, was very unsatisfactory and only about half that obtained with meal 1.

Mortality

In recording the mortality of chicks during the four cod liver meal experiments, no record was made of the chicks removed at the 21st, 35th, and 56th day for examination of the tibiae. The following table reports the date and number of mortalities which occurred during the experiments under discussion.

Table II

Mortality Record

Test 1	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	Total
2.00% Meal.....	2	—	—	—	—	—	—	—	2
0.50% C.L.O.....	—	—	—	—	—	—	—	—	—
Test 2									
2.00% Meal.....	—	—	—	—	—	—	—	—	—
0.50% C.L.O.....	—	—	—	—	—	—	—	—	—
Test 3									
2.00% Meal.....	—	—	—	—	—	—	—	—	—
0.25% C.L.O.....	1	—	—	—	—	—	—	—	1
Test 4									
2.00% Meal.....	—	1	—	—	—	—	1	4	6
0.13% C.L.O.....	1	—	—	—	—	—	—	2	3

From the above table, it will be noted that the mortality was twice as large for the cod liver meal as for the cod liver oil chicks. This result agrees with that obtained by Stuart (2) who reports twice the mortality for the chicks fed 2 per cent cod liver meal in the basal ration as for a comparable pen fed cod liver oil as a vitamin supplement. Somewhat similar mortality was reported by Mussehl (3) and co-workers who lost 47.5 per cent of the birds fed cod liver meal as a supplement to their basal ration, 62.5 per cent of the birds receiving the basal ration unsupplemented, and only 5.0 per cent of the birds which received cod liver oil as a supplement to the basal ration.

Bone Development

While space does not permit the inclusion of photographs of all the tibiae removed from chicks during this investigation, it is felt that it will

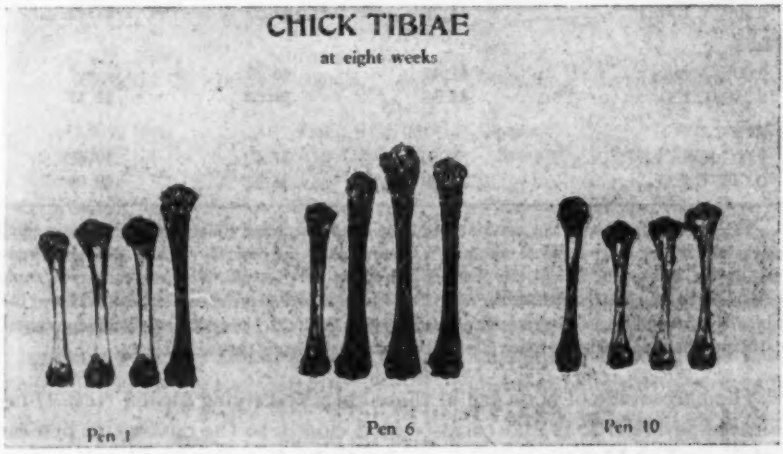

Fig. 1.

assist the reader better to visualize the extent of bone development obtained if a photograph of one series of tibiae is presented. Accordingly, the accompanying photograph (Figure 1) includes, tibiae from the negative control pen (pen 1) which received the basal ration unsupplemented, tibiae from the positive control pen (pen 6) which received the basal ration supplemented with 0.13 per cent cod liver oil, and tibiae from the experimental pen (pen 10) which received the basal ration supplemented with 2.00 per cent cod liver meal 4. The differences in development of the tibiae represented are very evident to the eye.

At the termination of the third, fifth, and eighth weeks, four representative chicks from each pen were killed. Both legs were dissected and freed of adhering tissue. For the ash determination, the left tibiae were dried for 48 hours at 96° C., crushed and extracted with hot alcohol (95 per cent) for 24 hours, weighed and ashed in an electric muffle furnace. The data obtained are recorded in Table III.

TABLE III

AVERAGE PERCENTAGE OF ASH IN THE LEFT TIBIAE (FAT FREE)

Test 1	At Three Weeks	At Five Weeks	At Eight Weeks
2.00% Meal	44.78	43.32	48.43
0.50% C.L.O.	42.02	49.27	48.55
Test 2			
2.00% Meal	44.47	44.98	46.63
0.50% C.L.O.	44.20	47.35	47.42
Test 3			
2.00% Meal	41.41	46.27	—*
0.25% C.L.O.	43.35	39.63	42.17
Test 4			
2.00 Meal	38.56	37.60	39.82
0.13% C.L.O.	37.49	36.92	40.09

* The determination of the ash content of the dried, extracted tibiae from the experimental chicks in test 3 at eight weeks of age, gave a value of 26.51 per cent. Obviously, this value is unusually low, but by the time it was obtained the chicks were too old to be used for a redetermination of the ash content of the tibiae. The histological report of the extent of calcification of the tibiae is more or less in accord with an ash content of 26.51 per cent, *i.e.*, "wide epiphyseal cartilage, abnormal marrow, space filled with cancellous osseous tissue."

The percentage of bone ash of those chicks receiving meal 4 (test 4) indicated severe rickets and corresponded closely to the oil control pen receiving only 0.13 per cent cod liver oil. Meal 3 (test 3) gave slightly better results, agreeing with the control pen receiving 0.25 per cent cod liver oil, although there was a decided drop in the percentage of ash between the fifth and eighth weeks. The percentages of ash in the tibiae from chicks which received meals 1 and 2 (tests 1 and 2) were fairly comparable and approximately that of the control pens which received 0.50 per cent cod liver oil. In general, the ash results were in accord with the growth curves.

CALCIFICATION

To obtain a more complete knowledge of the antirachitic value of the cod liver meals, the extent of calcification was determined by a modification

of the line-test method as described by McCollum (4) and his co-workers. The right tibiae, taken from test chicks on the 21st, 35th, and 56th day of the test, were freed of adhering tissue and immersed in 10 per cent formalin for 24 hours. They were then washed and longitudinal sections were cut from the proximal end. The sections were treated with 2 per cent silver nitrate and intensified under bright light until the calcified area became dark brown, indicating the degree of calcification. The results are recorded below.

TABLE IV

DEGREE OF CALCIFICATION

Basal Ration supplemented with	At Three Weeks	At Five Weeks	At Eight Weeks
	Test 1		
2.00% Meal	Normal	Normal	Fair
0.50% C.L.O.	Abnormal	Normal	Normal
	Test 2		
2.00% Meal	Normal	Normal	Normal
0.50% C.L.O.	Normal	Normal	Normal
	Test 3		
2.00% Meal	Fair	Poor	Very poor
0.25% C.L.O.	Abnormal	Abnormal	Poor
	Test 4		
2.00% Meal	Poor	Poor	Very poor
0.13% C.L.O.	Poor	Poor	Very poor

The results show that meal 2 (test 2) was superior to all the other meals for promoting calcification. Apparently cod liver meal 2, when used in conjunction with the relatively high vitamin ration employed in this study contained sufficient antirachitic vitamin to protect chicks from rickets for a period of eight weeks. Meals 3 and 4 (tests 3 and 4) possessed the poorest calcifying properties and afforded little if any protection against rickets, while meal 1 (test 1) assumed a midway position, affording protection for only about five weeks.

DISCUSSION

From the foregoing data it will be noted that the four samples of cod liver meal differed significantly in their vitamin value. Judging from all the results obtained, namely; rate of growth, tibial percentage of ash, and deposition of calcium, meal 2, having an antirachitic value equivalent to 0.50 per cent cod liver oil, was far superior to meals 3 and 4. Perhaps one would have anticipated this result since cod liver meal 2 contained the

highest percentage of fat, but Bethke (5) and co-workers report that, "The antirachitic variation is not proportional to the residual fat content of the livers." This conclusion was confirmed in the results of the test with cod liver meals 1 and 3 (tests 1 and 3) which have practically the same fat content, but which produced decidedly different growth and bone development.

Meals 3 and 4 (tests 3 and 4) were decidedly the poorest. The growth and physical condition of the chicks were extremely poor. They exhibited bad cases of leg weakness, staggering gait, lack of control and spasms of trembling, all of which, according to Cruickshank (6) and co-workers, may have been indications of lack of vitamin A.

Thus it is apparent that the vitamin content of cod liver meal is extremely variable and the meal may be of little if any value as a source of vitamin D. This conclusion is in accord with the findings of Bethke (5) and coworkers who report that "it would seem unwise to use the liver meal as an antirachitic substitute for a good grade of cod liver oil in either poultry or livestock production." The results of this investigation are also in harmony with those reported by Mussehl, *et al;* (3) who conclude that "cod liver meal made entirely from dried ground liver tissue contains some vitamin D, but not as much as is associated with an equal amount of fat in cod liver oil." These results are not in accord with those reported by Stuart (2) who states "cod liver oil and cod liver meal were quite comparable in relation to growth." Cruickshank, *et al*; (6) have also studied the vitamin A value of cod liver meal but their findings are not in agreement with those of Stuart for they report 10 per cent of cod liver meal was not sufficient to protect chickens against vitamin A deficiency.

Summary

Samples of cod liver meals were obtained on the open market. These were added to a typical poultry ration. Pens of twenty Rhode Island Red baby chicks were used for the test. Control pens received cod liver oil incorporated in the basal ration. The experimental period was of eight weeks duration and test birds were removed from each pen at the end of the third, fifth, and eighth week. These were killed, and the tibiae removed and examined to determine the extent of calcification. It was found that the best cod liver meals produced about the same results when 2 per cent was added to the basal ration as when one-half of one per cent of cod liver oil was added. The poorer meals were found to possess little if any vitamin value.

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A TENTATIVE METHOD OF ASSAYING FOODS FOR VITAMIN G*

By

HAZEL E. MUNSELL

(From the Bureau of Home Economics, United States Department of Agriculture, Washington, D.C.)

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RECOGNITION of the fact that many of the effects formerly ascribed to vitamin B as a single factor were due to one or more associated factors has made necessary further study of assay methods. The early work on the differentiation of vitamins B and G (3) clearly shows that vitamin G has growth-promoting properties.

Methods for quantitatively comparing foods as sources of other growth-promoting vitamins have been made dependent upon the growth responses of suitable laboratory animals to measured amounts of a test food when fed as the sole source of the vitamin in question. These methods require a basal diet that is free from the vitamin to be tested. In the application of this method to vitamin G determination, a difficulty is encountered in the fact that no convenient food has been found that will supply to the basal diet an adequate amount of vitamin B without admixture of vitamin G. Consequently it has been necessary to prepare extracts of a food rich in vitamin B and low in vitamin G using a solvent which removes only minimum amounts of vitamin G. The preparation of such extracts involves much time and expense and many laboratories are not in a position either to prepare or to purchase them.

Goldberger (4) stated that, in preparing vitamin B concentrates, maize might serve as a better initial source than yeast since it was rich in vitamin B and relatively poor in the associated thermostable factor (vitamin G). Furthermore, in the summary of work on vitamin B in wheat and corn, Hunt (5) states that Croll and Mendel found 20 to 30 per cent of corn an adequate source of vitamin B.

It was the purpose of the present investigation to ascertain if, in the assay of vitamin G, white corn could be used to supply the necessary vitamin B.

* Read before the meeting of the American Chemical Society at Atlanta, Georgia, April 8–11, 1930.

Experimental

White Corn as a source of vitamin B relatively free from vitamin G. The basal diet 107 of Sherman and Spohn (10) composed of purified casein, 18 per cent; starch, 68 per cent; butter fat, 8 per cent; cod liver oil, 2 per cent; and Osborne and Mendel salts, 4 per cent, contains no detectable amounts of vitamin B or vitamin G.

In order to test the adequacy of white corn as a source of vitamin B and also to ascertain the relative amount of vitamin G present, six modifications of this diet were prepared. In the first three, corn replaced the starch in the basal diet to the extent of 10, 20, and 30 per cent respectively, and was the only source of vitamins B and G. For convenience these diets are designated as 107–C10, 107–C20, and 107–C30. The other three diets were similar to these except that each contained 10 per cent of autoclaved yeast in place of an equivalent weight of starch. The yeast was autoclaved by placing it in petri dishes in layers ¼ inch thick and heating in an autoclave for 4 hours at 15 pounds pressure. Many tests carried out in this laboratory have shown that this procedure destroys any detectable amounts of vitamin B in the yeast. Thus in these three diets designated respectively as 107G–C10, 107G–C20, and 107G–C30, the corn continued to remain the sole source of vitamin B, while vitamin G was adequately supplied by the autoclaved yeast with corn as a second possible source.

Six groups of 28-day old rats were then given one each of these diets for a period of eight weeks. Chart 1 shows the results obtained with diets 107G–C10, 107G–C20, and 107G–C30. It is evident that neither 10 nor 20 per cent of white corn supplies an amount of vitamin B sufficient for normal growth under the conditions of this test, since 10 per cent did not make it possible for the rats to maintain their original weight and 20 per cent supported a gain of only 59.5 grams during the 10 weeks. However, 30 per cent of corn supplies sufficient vitamin B for normal growth during this period, since the rats on diet 107G–C30 gained 101.5 grams in the 8 weeks of the test. The intake of corn averaged 0.29 grams per rat per day on diet 107G–C10, 1.18 grams on diet 107G–C20, and 2.5 grams on diet 107G–C30.

Chart 2 gives the results with diets 107–C10, 107–C20, and 107–C30. From these results it can be seen that white corn contains very little vitamin G since the average gain in weight of the group of rats on diet 107–C30 was only 7 grams while on diets 107–C10 and 107–C20 the rats lost weight. That vitamin G and not vitamin B was the first limiting factor for growth is shown by comparing the average daily corn intake of the two groups of animals portrayed in Charts 1 and 2. In Chart 1 it will be noted

that 0.29 and 1.18 grams of corn per rat per day provided a source of vitamin B which was responsible for gains in weight of −4.5 and 59.5 grams respectively. In Chart 2, 0.27 grams and 1.21 grams of corn supplied both vitamins B and G and the respective gains in weight were −13.0 grams and 7.0 grams. In other words a relatively smaller amount of corn is required to furnish an amount of vitamin B for a given gain in weight than is required to furnish vitamin G sufficient for the same gain in weight.

Other tests were then carried out in order to further substantiate the

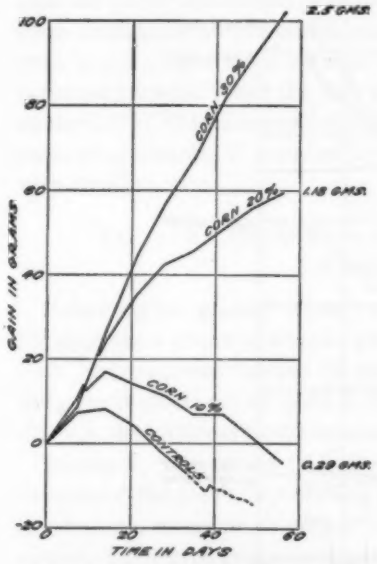

CHART 1. Curves showing average changes in weight of groups of rats fed a diet in which white corn was the sole source of vitamin B. The per cent of corn is indicated on the respective curves and the average intake of corn in grams per rat 6 times per week is given at the end of the curve.

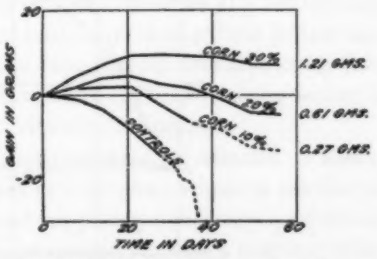

CHART 2. Curves showing average changes in weight of groups of rats fed a diet in which white corn was the sole source of vitamin B and vitamin G. The per cent of corn is given on the respective curves and the actual intake of corn in grams per rat six times per week is given at the end of each curve.

evidence that corn contains very little vitamin G. The results obtained by Mitchell (6) and later by Goldberger (4) using alcohol extracts of white corn as a source of the antineuritic vitamin indicated that vitamin B is readily soluble in alcohol of all concentrations up to 80 per cent by weight, and that vitamin G is decreasingly soluble with increasing concentration. Later, the work of Sandels (8) confirmed this impression. Accordingly, an

alcohol extract of white corn should contain the vitamin B originally present without admixture of significant amounts of vitamin G.

An 80 per cent (by weight) alcohol extract of white corn was prepared, evaporated down on cornstarch and tested for its vitamin B and G content. It was first tested for vitamin B by feeding at levels of 2, 3, and 5 per cent[1] replacing an equivalent weight of starch in diet 107G (diet 107 with 10 per cent autoclaved yeast in place of an equal weight of starch). The results of

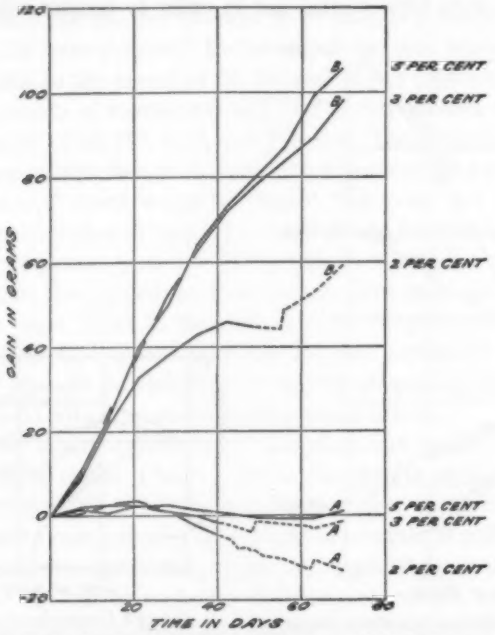

CHART 3. Curves showing change in weight of six groups of rats. Those designated by "A" received an 80 per cent (by weight) alcohol extract of white corn as the sole source of vitamins B and G. Those designated by "B" received the extract as the sole source of vitamin B. The percentage of extract included in the diet is indicated at the termination of the curve.

these tests are shown by the curves marked B in Chart 3. At a level of 2 per cent the extract furnished enough vitamin B for fair growth for the first 40 days, after which there was a decline. Three and five per cent furnished sufficient vitamin B for normal growth.

[1] These percentages are based upon Goldberger's units in which 1 per cent is equivalent to the extract obtained from 18 per cent corn.

The vitamin G content of the extract was determined by feeding it at levels of 2, 3, and 5 per cent in diet 107 thereby allowing the extract to be the only source of vitamin B and G available to the animals. Having already ascertained that as little as three per cent of the extract was an adequate source of vitamin B, any growth in this case would necessarily be due to the vitamin G that it supplied.

The curves marked A in Chart 3 summarize the results obtained. The increase in growth responses of the groups of rats was not proportional to the increase in the percentage of the extract used which would indicate that the extract contained little or no vitamin G. If this is true then rats on diets containing no vitamin G maintain weight at or only slightly below their initial weight for some time and in most cases survive the usual test period of 8 weeks. When the very slight gain in weight of the rats (Chart 2) on diet 107–C30 is compared with these results it is again apparent that the amount of vitamin G supplied by 30 per cent of white corn in the diet is very small.

Tentative Method for the Assay of Foods for Their Vitamin G Content

Following the general vitamin assay methods based on the relationship between the amount of vitamin present and the rate of growth in test animals, Sherman and Sandels (9) reported experiments demonstrating that the growth produced by diets in which vitamin G is the limiting factor, is directly proportional to the amount of vitamin G supplied.

Bourquin, attempting to find a method suitable for vitamin G assay, considered the possibility of using 20 per cent of whole wheat in the diet as an adequate source of vitamin B without supplying significant amounts of vitamin G. She fed graded amounts of autoclaved yeast as a source of vitamin G in addition to a basal diet containing 20 per cent of whole wheat as the only source of vitamins B and G. However, at the usual level of gain of 3 grams per week, the responses were so irregular that conclusions could not be drawn with any degree of certainty. For this reason she discounted the possibility of using whole wheat as the source of vitamin B in an otherwise vitamin G-free basal diet and resorted to the use of alcohol extracts of wheat.

The work reported here was practically completed before Chase (2) and Bourquin (1) made available their results relative to the amounts of vitamins B and G in wheat. Chase found that a daily supplement of 1.0 gram of whole wheat per rat per day as the sole source of vitamin B induced an

average gain in weight of 59.7 grams in 8 weeks. The results in Chart 1 show that 1.18 grams white corn supported an average gain of 59.5 grams in 8 weeks. This would indicate that whole wheat is slightly richer than corn in vitamin B. The work of Mendel and Croll (5) has shown them to have about equal value.

Bourquin (1) found that 0.2 gram of whole wheat per rat six times per week as the sole source of vitamin G resulted in an average gain in weight of 6.6 grams during 8 weeks. In our tests 1.21 grams of corn as the sole source of vitamin G induced an average gain of 7 grams during 8 weeks, showing that white corn contains less vitamin G than wheat.

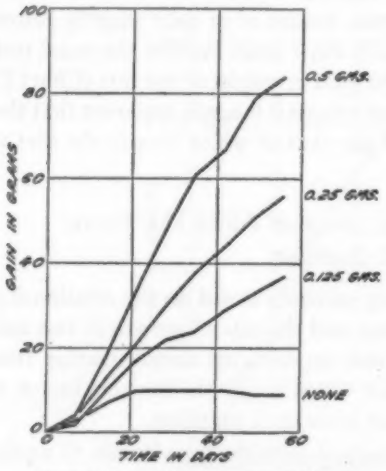

CHART 4. Curves showing average changes in weight of groups of rats fed graded portions of autoclaved yeast as the source of vitamin G. The basal diet used contained only the small amount of vitamin G present in the 30 per cent of white corn used as a source of vitamin B. The amount of autoclaved yeast fed each rat 6 times per week is given in grams at the end of the curve.

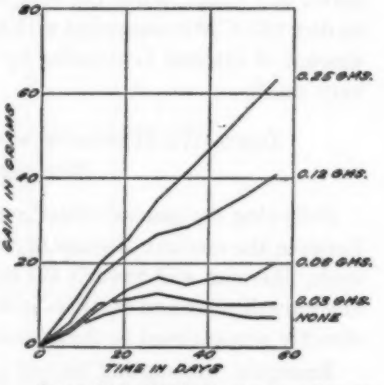

CHART 5. Curves showing average changes in weight of groups of rats fed graded portions of vegetable extract as the source of vitamin G. The basal diet used contained only the small amount of vitamin G present in the 30 per cent of white corn employed as a source of vitamin B. The amount of vegetable extract fed each rat 6 times per week is given in grams at the end of each curve.

Since our results showed that 30 per cent of corn in the vitamin B-free diet of Sherman and Spohn supplied an adequate amount of vitamin B, diet 107–C30 was tested as a possible basal diet to be used in determining the vitamin G content of foods. A number of foods have been tested and the results obtained with autoclaved yeast and a vegetable extract are given here. In Chart 4 are shown the average gain-in-weight curves of

groups of rats fed graded portions of the autoclaved yeast as the sole source of vitamin G. The amounts used were 0.125, 0.25, and 0.5 gram per rat 6 times per week and the corresponding gains in weight were 36.5 grams, 55.5 grams, and 83.8 grams. In Chart 5 are given the results obtained when graded quantities of a well-known vegetable extract supplied the sole source of vitamin B. The amounts fed were 0.03, 0.06, 0.12, and 0.25 gram per rat 6 times per week and the corresponding gains in weight were 10.5, 20.0, 40.5, and 63.5 grams respectively. In the latter case the growth response was very evenly graded to the dose. This product contains a fair amount of vitamin B which may in part account for the uniform results obtained.

These results show that a diet containing 30 per cent of white corn as the sole source of vitamin B may be used in determining the presence and the relative amount of vitamin G present in a foodstuff. It is of course necessary to take account of the fact that small but minimal amounts of vitamin G are present in the corn. This can be done by comparing all results with those obtained with the control animals on diet 107–C30 as a base.

Summary and Conclusions

1. Results of tests with groups of white rats show that 30 per cent of white corn in the Sherman and Spohn vitamin B-free diet does not supply an amount of vitamin G sufficient to promote growth or to prevent the occurrence of symptoms of pellagra.

2. Normal growth results when 30 per cent of white corn is fed as the only source of vitamin B in the Sherman and Spohn diet to which autoclaved yeast has been added to supply vitamin G.

3. An 80 per cent (by weight) alcohol extract of white corn fed at a level of 3 per cent supplies an amount of vitamin B sufficient for normal growth, and contains very little vitamin G. These tests also indicate that rats on a diet free from, or at least containing only minimal amounts of, vitamin G, tend to maintain weight for some time at or near their initial weight. This gives further evidence that the diet carrying 30 per cent of white corn contains very little vitamin G.

4. On a diet in which the vitamin G carried by 30 per cent of white corn was the limiting factor, growth of young rats is shown to be proportional to the vitamin G of the test food fed in addition to this basal diet. Results of tests with autoclaved yeast and with a vegetable extract are given.

5. In cases where conditions do not permit of the preparation or purchase of vitamin B extracts for use in vitamin G assays, white corn to the

amount of 30 per cent of the diet may be used to supply an adequate amount of vitamin B with little addition of vitamin G. When this diet is used all comparisons must be made relative to the growth rate of the controls on the basal diet only.

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THE EFFECT OF PASTEURIZATION UPON THE VITAMIN C CONTENT OF MILK IN THE PRESENCE OF CERTAIN METALS*

By

E. W. SCHWARTZE, F. J. MURPHY AND GERALD J. COX

With the assistance of William Waugh, Elizabeth Clark, and Mary Marsh.

(*From the Mellon Institute of Industrial Research,† Pittsburgh, Pa.*)

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INTRODUCTION

THIS report deals with a study of the effect of certain metals upon the destruction of the antiscorbutic vitamin during the aerobic pasteurization of milk. The data obtained not only are of value in indicating the potentialities of the metals that were studied, but also are probably indications of the percentage of destruction of vitamin C which will occur in the aerobic pasteurization of fresh summer milk.

The problem before the dairy industry today regarding the preservation of the antiscorbutic vitamin in milk may be summed up as one in the combatting of oxidation. Time, temperature, and the repeated contact of air with milk are factors that influence this action. The metals used in dairy equipment also contribute because of the possible catalytic rôle that they may play in oxidation. Metals may also be objectionable if they have appreciable solubility in the milk, or impart a flavor (1). But it is practically axiomatic in the dairy industry that most equipment must be metallic on account of the necessity for good heat conduction as well as for durability. It is obvious, therefore, that an acceptable metal must meet both rigid physiological and mechanical requirements for the maintenance of an unimpeachable position.

Our experiments have been confined to the comparison of copper, tinned copper, and aluminum continuous flow pasteurizers because there is a tendency toward the use of this type of pasteurizer on account of its anaerobic features. For strict comparison of the metals, block tin might have been used instead of tinned copper, but such equipment is practically unknown both on account of the softness of the metal and its high cost.

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Most of the metallic dairy equipment in use at present for processing milk is constructed of tinned copper, except vacuum pans for condensing milk, which are made of naked copper. It may be stated, moreover, that considerable laxity exists in retinning of equipment when worn. Copper is decidedly objectionable in dairy practise owing to the taste it imparts to the milk.

Copper oxide surface is more soluble in milk than bright metallic copper surface (2). Hess and Weinstock (3) have shown that destruction of vitamin C is catalyzed by metallic copper. Keeping the copper bright by constant polishing or use is, therefore, only a partially palliative measure. No direct evidence is available concerning the effect of tin, but presumably from the degree of preservation of vitamin C in some canned vegetables, notably those of an acidic nature such as tomatoes (4), it does not appear that tin itself should be directly under suspicion. Schwartze, Murphy, and Hann (5) have shown that, if hard glass (pyrex) may be taken as a standard, aluminum is also inert and has no deleterious effect upon vitamin C in the boiling of milk. No report of a comparison of copper, tinned copper, and aluminum is available in the literature. The qualities of various metals including tin, copper, and aluminum, from other standpoints of the dairy chemist have, however, been discussed by Hanziker, Cordes, and Nissen (1).

The excellent heat conductivity of aluminum, its low initial and maintenance cost, its ease of fabrication, light weight, and durability[1] are factors that eminently recommend it from an economic viewpoint for use in the dairy industry. In our experience aluminum does not impart a flavor to milk and our analyses indicate that it is not noteworthily corroded by contact with milk. This study of the stability of vitamin C in the pasteurization of milk in aluminum has been proposed by one of us (E. W. S.) to demonstrate the degree of suitability of the use of aluminum in the dairy industry from a public health point of view.

Experimental

The vitamin C destruction in milk samples pasteurized respectively in aluminum, tinned copper, and copper has been determined quantitatively by measuring their values in preventing scurvy in guinea pigs.

The animals subsisted on a scorbutic diet and graded supplementary doses of milk. Six groups of animals were used, as follows:

[1] The pitting of aluminum formerly observed can now be obviated (6) and non-corrosive alkaline cleansers are also available. There is no necessity for the employment of chlorine-containing disinfectants in a modern dairy plant because steam will sterilize if properly used.

a. A negative control group received no supplementary milk.

b. An autopsy control group was fed spinach as a source of vitamin C.

c. A standard comparison group received graded doses of raw milk.

d, e, f. Three groups received graded doses of milk pasteurized respectively in aluminum, tinned copper, and copper.

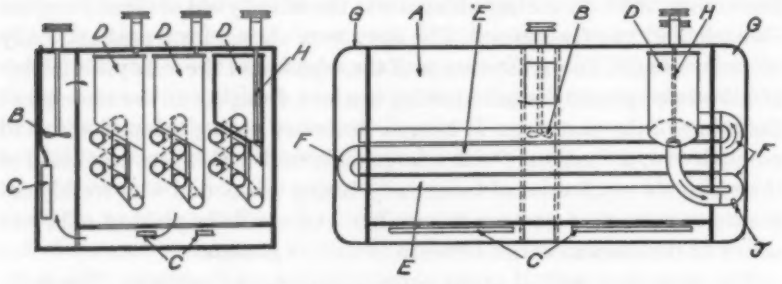
Milk supply. The milk used for this experiment was of certified quality and was obtained from the Braeburn Farms of the Liberty Dairy Products Corporation of Pittsburgh between the middle of May and the middle of September, 1929. At the beginning it was the mixed yield of three cows(one Holstein and two Guernseys). The cows were chosen for a medium, daily quantity of milk. In the later stage of the experiment the milk yield fell below the daily quantity required owing to a long drought and the consequent drying up of the pasturage. It became necessary during this period to add a fourth cow, a Guernsey, with a large daily milk yield. The usual feed of the cows was supplemented twice daily during this period with freshly cut greens or oats, peas and soy beans. The average daily yield of milk was about 18 pounds and varied between 14 and 23 pounds.

The cows were milked about three o'clock in the morning. The milk, after being cooled and bottled to conform to the requirements of a certified dairy, was shipped in ice-packed cases by express to Pittsburgh. It was delivered to the laboratory by truck about 9 a.m. By this method milk was obtained ready for use six hours after the cows were milked. The feeding of the guinea pigs was begun immediately after pasteurization of the milk in the laboratory and all feeding was completed shortly after 6 p.m. The statistical age of the milk as fed, therefore, was about 12 hours.

No facts sufficiently precise are known concerning the comparative qualitative value of pasturage and ensilage in producing milk of high antiscorbutic property. The literature, and our past and present experiment, would tend to indicate that ensilage has been generally superior. The circumstance of a possible depression of vitamin C concentration because of a failure of pasturage was provided for by control experiments conducted simultaneously.

Pasteurizing equipment. The milk was pasteurized in a specially constructed laboratory apparatus (Figure 1). It was designed with a view to obtaining exactly parallel conditions in three pasteurizers each of which was constructed of a different metal. They were contained in a large constant temperature bath (A). The water in the bath was circulated by means of a motor-driven propeller (B). Heat was supplied by electric heaters (C) from the bottom and also from the side close to the propeller of the bath.

Each pasteurizer consisted of a chamber (D) and two long tubes (E) connected together in one closed system and entirely contained within the constant temperature bath with the exception of the connecting bends (F). These bends were outside the bath but were protected from the surrounding atmosphere by removable insulated shields (G). The bends were themselves removable, being held in place by short pieces of rubber tubing. The apparatus was so constructed to facilitate cleaning. Each pasteurizer was equipped also with a motor-driven screw-type propeller (H) of the same



Continuous Flow Pasteurizer

FIGURE 1.

metal as the pasteurizer to keep the milk circulating actively during pasteurization. Care was taken in the use of these propellers to see that the milk was not unnecessarily churned or beaten. A small rubber stoppered hole (J) was provided at the lowest point in each pasteurizer for draining.

Meticulous care was taken in cleaning the pasteurizers to maintain a bright surface throughout at all times. After each pasteurization a hot 5 per cent solution of non-corrosive alkaline cleanser[2] was circulated in the pasteurizer for 15 minutes. After this solution was drained, the tubes and chambers were thoroughly scrubbed with stiff dairy brushes and tap water. Fine steel wool and soap were used to clean all available surfaces but with a sparing use on the tinned copper. As the use of the steel wool gradually removed the tin coating about the circumference of the ends of the tubes of the tinned copper pasteurizer, they were repeatedly retinned with block tin so that a higher standard of maintenance was observed than in ordinary dairy practise on this type of equipment. The extent of corrosion of the aluminum and copper pasteurizer due to the cleaner[2] which contains an inhibitor or anti-catalyst for aluminum corrosion, is shown in Table I.

[2] Oakite Multicleaner.

TABLE I
ANALYSES OF MILK FOR ALUMINUM AND COPPER

	Pasteurization in Aluminum		Pasteurization in Copper	
	Before p.p.m.	After p.p.m.	Before p.p.m.	After p.p.m.
Milk	0.07	0.67	—*	7.2
			—*	8.6
Cleaner solution	0.08	0.41	0.02*	8.2

* No exact quantitative significance is attached to the figures obtained as they averaged less than 0.1 p.p.m. The previous limits of accuracy of the method do not seem to have been proven, and milk runs lowest of all food products reported (0.15 p.p.m. Cu).

Pasteurization of milk. The milk was pasteurized immediately after it was received from the dairy. The milk, at about 8°C., was transferred from the bottles directly into the pasteurizers. Three quarts of milk were used in each pasteurizer. The temperature of the bath had previously been brought to 61 to 62°C. Circulation was started in the pasteurizers immediately. A preliminary pre-heating period of about ten minutes was required to bring the milk to approximately the pasteurizing temperature. This brought the temperature usually to 58.5 to 59.5°C. The pasteurization was timed for 30 minutes from this point. The temperature usually rose to 60°C. or above in the first five minutes of the pasteurizing period and varied from 60.0 to 60.5°C. during the remainder of the process.

It was found that there was a lag of one to two minutes in the temperature rise of the milk in the aluminum pasteurizer. This pasteurizer was, therefore, filled first and emptied last, making the total time of milk in the pasteurizer two to three minutes longer than in the tinned copper and copper pasteurizers. In this way the lag was compensated for and, incidentally, the aluminum was given a slightly more severe test than either of the other metals. The order of filling and emptying the tinned copper and copper pasteurizers was alternated daily so that as between themselves these two metals were given equally severe tests.

When the pasteurizers were emptied, the milk was run directly into ice-packed pyrex glass flasks. It was cooled to below room temperature in these flasks in about five minutes. It was immediately bottled and capped and placed in the refrigerator until used.

The data of the analyses for aluminum of raw milk and of milk pasteurized in aluminum and also those for copper in raw milk and milk pasteurized in copper are shown in Table I.

Selection and maintenance of experimental animals. Young male guinea pigs weighing 200 to 240 grams were purchased from a local breeder. They were placed in individual cages and fed the basal ration (Table II), 35 grams of fresh spinach and 10 to 30 cc. of fresh whole milk daily. This diet differs essentially from that usually employed in scurvy experiments in that it contains no heated skim milk powder. It is equally as efficient in protein as the heated skim milk powder diet and is superior on account of ease of preparation and possibly on account of a better supply of other vitamins(7) This diet supports excellent growth when fed with greens. The body weight and food consumption were watched for 10 to 20 days as well as the condition and type of respiration. Weaklings and those animals showing signs of respiratory disease, which is endemic among commercial guinea pigs, were eliminated. Owing to the failure of complete elimination of undesirable animals within the preliminary period, it was necessary to discard a few animals very early in the experiment proper. Considerable success was had in getting desirable animals, although the percentage of rejections was sometimes high.

The rejected animals were subjected to autopsy for the purpose of checking the quality of the animals purchased. Out of 132 examined, four animals had slight macroscopic lesions of the ribs, presumably scorbutic. The occurrence of traces of scurvy in rejected animals was very probably greater than that in the animals retained. The effect of an occasional animal showing traces of old scurvy, inadvertently introduced into the experment, was minimized by our use of a large number of experimental animals.

Milk was fed by pipette both during the preliminary and experimental periods. This method has an advantage over cup feeding as it insures a constant daily dosage, avoids exposure with a consequent depression of

TABLE II

COMPOSITION OF THE BASAL RATION*

	grams
Ground rolled oats	500
Ground bran	300
Peanut meal	100
Filtered butter fat	49.5
Cod liver oil	0.5
Dried brewer's yeast	20
Salt mixture**	25

* Crude protein, 19.6 per cent. Calcium, 0.565 per cent. Phosphorus, 0.835 per cent.

** Salt mixture, 4.1 grams mono-sodium phosphate, 8.4 grams sodium chloride, 12.5 grams calcium carbonate.

vitamin C content of the milk and prevents the consumption of cream layers. The same type of cages and the same laboratory routine were employed as in former experiments (5). Animals and food were weighed every third day, and fresh trays were supplied.

Attention is directed to examination of the lungs at autopsy. Since our earlier report upon our efforts to obtain experimental animals free from lung complication went to press, and at the conclusion of the experiments herein reported, the work of Tyson and Smith (8) upon chronic lung infection in rats, as well as the work of Coryllos, *et al.;* Henderson *et al.* (9), on dogs appeared. Their work is completely in harmony with our inference from gross pathological examination of lungs of guinea pigs that atelectasis precedes or predisposes to an infection. The effect of insufficient vitamin C in the guinea pig is to restrain it and therefore to increase the effect of the already too potent element of confinement present in any experiment. This factor is very disturbing at times and must be adequately ruled out by application of vigorous methods, or its presence rigorously controlled. Failure to report this condition in the course of experimentation cannot be accepted as evidence of its non-existence in other scurvy work.

Negative controls. The composition of the basal diet is given in Table II. The results of feeding the diet are given at the bottom of Table IV. These experiments indicate that the diet was sufficiently free (possibly entirely free) from vitamin C for use for this purpose. Attention is therefore called to the ease with which a successful scurvy-producing diet may be made from raw materials readily at hand, and without recourse to excessive and questionable devitaminization procedures.

Autopsy controls. The autopsy control animals were fed the basal diet and 15 to 20 gms. of spinach daily. They outgrew the best of the animals receiving supplementary antiscorbutic vitamin only from milk. This speaks for the existence of a considerable range between the apparent protective and the optimum vitamin requirement as well as showing that these animals fed milk only could not be considered as positive controls in the strict sense.

Criteria of assay. The criteria of assay of vitamin C destruction depend upon the production of equal or approximately equal degrees of effect (*i.e.*, scurvy) in respective series of animals from definite doses of milk prepared in different ways. The equality or practical equality was judged by three criteria, namely, clinical course of the scurvy, pathological findings upon post mortem examination, and growth. In biological assay it is necessary to employ a large number of experiments on the same dose to estab-

lish an average. It is also advisable to confirm a conclusion by the use of series averages at other levels of experimentation.

Five stages of clinical scurvy were recognized in addition to the free or doubtful group. It is probably true that many animals diagnosed as free could have been shown by microscopical examination of the tissues to have incipient scurvy. There is no evidence that going through this procedure would have increased the accuracy of the assay. It would have tended to make it unduly involved.

The five clinical stages of scurvy are evaluated, respectively, in the tables by numbers from one to five. The first stage was shown by tender wrists, or tender and swollen wrists. In the second stage, swelling and tenderness of the hind legs appeared, together with the wrist symptoms. In the next stage, "3" in the tables, there was some marked impairment of function of the limbs and the appearance of the scurvy position. In the fourth stage, the hind limbs had become practically functionless, and there sometimes occurred gastro-intestinal symptoms such as prolapse of the rectum or hemorrhage. In the last stage, the animal could hardly feed itself and would fall after a short effort to stand; in other words, it was in or near the moribund state. There was, of course, some deviation in the way in which different animals in the same experimental group reacted as well as the manner in which we were occasionally forced to apply the classification scheme in border-line cases. The five clinical stages of scurvy in addition to a free stage were easily recognized pathologically.

A pathological examination was made on every animal. The jaw and joints (knee) were examined for fragility, the teeth, especially lower incisors, for looseness, and the ribs for beading. The ribs, intestines, joints (knee), and muscles were examined for hemorrhages and oedema. The wrists, scored separately (see second column of tables under "Pathological'), were especially examined for swelling, hyperemia, oedema, signs of old hemorrhage, and overgrowth of bone or old scurvy. Three degrees of lesions were estimated, namely, mild, moderate, and severe, and only rarely intermediate or half stages. In adding up and averaging the numbers indicating the degree of the lesions, we are on insecure ground since there is no proof that fragility of bones, hemorrhage, or oedema should be given equal weight. We have not given, however, high statistical importance to averages, but used them for that which can be seen in a glance without involved computations. For one who has seen considerable scurvy in guinea pigs, it would be equally easy to establish different degrees (say about five) as we did in the clinical examination.

Time of experiments. The time between the beginning of the first and the end of the last experiment was about 5 months. The duration of the majority of the individual experiments was 70 days, and 40 days for a few groups on small amounts of vitamin which gave indications of not surviving as a unit for a longer period. In our experience 90-day tests do not seem to be essential, except possibly for bringing out or magnifying scorbutic states and lesions in animals apparently protected. Forty-day periods are not to be recommended, unless for some specific purpose, if the animals will survive longer as a group.

Results of Feeding Experiments

Raw milk. There are given in Tables III and IV the results of the experiments with the different dosages of raw milk. These experiments have been separated into two groups started at different times. The results indicate that 35 cc. of the raw milk were insufficient to protect guinea pigs from apparent clinical scurvy and that there was about a 20 per cent deterioration in the quality of the milk as the season advanced. The failure of 35 cc. of pasture-produced milk to protect in this instance was not unexpected. The deterioration in the quality of the milk was taken care of as the season advanced by running necessary comparisons simultaneously.

Milk pasteurized in copper. In Table IV are given the data showing the effect of pasteurization of milk in naked copper. Two copper series were run, the second one for confirmation of the striking deterioration of vitamin C as shown in the first. The results compared with those on 14 cc. of raw milk, which animals were killed at the end of 40 days, indicate that much more than 40 per cent of the vitamin C was destroyed. The results of experiments in which 3.5 cc. and 7 cc. of raw milk (diluted to 35 cc.) were fed are so close as to be indistinguishable from the experiments in which 35 cc. of milk pasteurized in copper were fed. We conclude that at least 80 per cent (if not actually 90 per cent) of the antiscorbutic vitamin was destroyed in pasteurizing in the naked copper pasteurizer. It should also be noted that these guinea pigs behaved very much like those receiving no milk. In placing the destruction at only 80 per cent, therefore, we are giving this milk the benefit of all doubt. These experiments confirm Hess and Weinstock (3) and also Flinn (10) as to the marked destructive effect of copper on vitamin C.

No feeding experiments with copper salts were conducted. The course of our experiments was that of typical scurvy and even though dietary copper were capable of producing symptoms and lesions similar to scurvy

TABLE III

THE ANTISCORBUTIC VALUE OF RAW MILK

Dose of raw milk per 300 grams body weight, cc.	Number of animals	Date of Beginning individual experiments	Duration of experiment	Observed Degree of Scurvy		Body Weight		
						Start	Maximum	End
				Clinical	Pathological			
		1929	Days			grams	grams	grams
35*	4	May 8 to June 10	70	1.4	2.0 and 0.5	294	522	508
35	8	July 14	70	2.75	4.0 and 0.75	288	460	434
28	7	May 8 to June 7	70	2.7	5.3 and 0.6	290	452	437
28	3	July 8	70	3.7	9.3 and 1.7	290	382	372
22.5	14	May 8 to June 10	70	3.1	8.3 and 0.6	298	418	382
17.5	2	June 10	70	3.0	14 and 1.5	288	357	297
17.5	6	July 8	70	3.8	13.8 and 2.5	299	369	339

* Divided into early and late experiments on account of slight deterioration of milk as season advanced.

TABLE IV

THE ANTISCORBUTIC VALUE OF MILK PASTEURIZED IN NAKED COPPER AS COMPARED WITH RAW MILK. NEGATIVE CONTROLS

Dose and Type of Milk per 300 Grams	Number of Animals	Date of Beginning	Duration of Feeding Milk*	Duration of Experiment*	Observed Degree of Scurvy		Body Weight		
							Start	Max.	End
					Clinical	Pathological			
		1929	Days	Days			grams	grams	grams
35 cc. Copper Pasteurized, 1st series	7	May 20	22	27 to 37 Av. 30	5.0	19.4 and 2.4	298	342	198
35 cc. Copper Pasteurized, 2nd series	16	July 28	19	24 to 35 Av. 28	4.4	18.0 and 2.4	298	324	177
14 cc. Raw milk	11	June 14	40	40†	3.3	10.4 and 1.3	284	354	297
7 cc. Raw Milk Diluted to 35 cc.	14	Aug. 7	19	22 to 36 Av. 28	4.4	19.2 and 2.7	299	319	181
3.5 cc. Raw Milk Diluted to 35 cc.	13	Aug. 4	19	20 to 37 Av. 29	4.3	19.0 and 2.25	294	319	183
Negative Control	17	Aug 5 and Aug. 10	0	17 to 35 Av. 24	3.9	16.3 and 2.4	294	311	171

* Animals fed as long as practicable as a group. Milk was then discontinued and experiments allowed to terminate spontaneously.

† All animals were living and were killed for autopsy at the end of 40 days.

(which it is not known to do) the elucidation of such a mechanism of its action is aside from the present purpose, and would, if it were true, not detract from the full significance of these data.

Milk pasteurized in aluminum and in tinned copper. In Table V are given the results of feeding milk pasteurized in aluminum and tinned copper. The autopsies on these animals were performed on a number at one time without knowledge of identity of the respective experiment. The animals were placed on the respective experiments without selection or any preference. The results when compared with those in Table III show between 20 and 40 per cent destruction of vitamin C. The results with the pasteurization in aluminum are more favorable than those with tinned copper both as regards the amount of clinical and pathological scurvy and the body weights. This conclusion was also reached with ease by watching daily the course of these experiments. The exact amount in favor of the aluminum could not be stated mathematically from series of this type without further experimentation. This observable difference is consistent with the findings on the naked copper experiment, inasmuch as evidence of slight wear was observed on certain parts of the pasteurizer inaccessible to tinning. These experiments also bear out our previous conclusion that there was some deterioration in the quality of the milk as the season advanced.

Conclusions

The effect upon the vitamin C content of pasteurizing fresh milk aerobically in aluminum, in tinned copper, and in copper tubular pasteurizers has been studied. The destruction of vitamin C was placed at 20 to 40 per cent in aluminum. This is greater than the destruction previously obtained upon boiling milk for 5 minutes and is rather to be expected as the duration of exposure in pasteurizing was much longer and strictly aerobic. No pasteurizing experiments were run in hard glass (pyrex) because equality of glass and aluminum had been previously established in the boiled milk experiments.

The effect of pasteurizing milk in tinned copper was slightly greater than that found with the aluminum. The difference is less than the order of the increments of dosage employed. The effect was noticeable both during the progress of the experiment and upon post-mortem examination of the animals. Each of the two series of experiments upon milk pasteurized in copper was run respectively and simultaneously with two series of experiments upon milk pasteurized in aluminum, so that possible deterioration of raw milk as the season advanced does not enter. This effect we believe

TABLE V

THE ANTISCORBUTIC VALUE OF MILK PASTEURIZED IN ALUMINUM AND IN TINNED COPPER

Dose and type of milk per 300 grams body weight	Number of animals	Date of beginning	Duration of experiments	Observed Degree of Scurvy		Body Weight		
						Start	Maximum	End
				Clinical	Pathological			
		1929	Days			grams	grams	grams
45 cc. Aluminum pasteurized	15	June 27 to July 3	70	2.5	4.6 and 1	291	502	486
45 cc. Tinned copper pasteurized	12	June 27 to July 3	70	3.6	7 and 1	288	435	415
35 cc. Aluminum pasteurized	7	May 20	70	2.3	5 and 1	302	450	438
35 cc. Tinned copper pasteurized	9	May 20	70	2.9	8.3 and 1.6	303	372	358

is referable (*vide infra*) to the small amount of copper exposed to the milk when in the tinned copper pasteurizer.

The pasteurizing of milk in naked copper resulted in a destruction of at least 80 to 90 per cent of the antiscorbutic vitamin, but since the exposure to copper as well as the aerobic pasteurizing process both tend to destroy the vitamin, all of the 80 to 90 per cent cannot be charged to copper. Since both acted simultaneously, attention is called to the fact that less than the stated 20 to 40 per cent must be charged to the aerobic pasteurization process itself because copper has the greater effect.

The practical importance of these experiments is that copper is again shown to be unsuited for construction of dairy equipment so far as the conservation of vitamin C is concerned. Tinned copper is likewise unsatisfactory both on account of the impracticability of replating of worn inaccessible parts of equipment and of the laxity in retinning by the dairy trade. Glass (pyrex) is assumed to represent an inert material and by previous experimentation with boiled milk no difference was found between it and aluminum. Aluminum is therefore as satisfactory a material as we have for the construction of dairy equipment as regards the destruction of vitamin C. We have reason to suspect that block tin would not be satisfactory.

From the standpoint of corrosion the position of aluminum as regards the extent of its entrance into the milk was satisfactory; 0.6 parts per million; normally present 0.074 parts per million. The objectionableness of copper is no longer a moot question even from the standpoint of the dairy chemists themselves, although small amounts—0.25 to 0.50 parts per million—may be normally present in milk. The amount of tin which was actually present is not known. Some undoubtedly wears off in cleaning. Tin and aluminum are both pharmacologically superior to copper for use as food containers. It is hard to conceive that hard glass (pyrex) would contribute substances (aluminum, etc.), to milk although the methods of analysis are not yet capable of answering this point. For the present, therefore, if one were to insist upon universal freedom of food containers and manufacturing equipment from corrosion, it must be sought in materials not now practical or available.

The experiments herein reported are not inconsistent with the theory that anaerobic pasteurization would reduce the vitamin destruction, possibly to zero, even in the case of copper. It should be pointed out in this connection that even though the vitamin destruction were accordingly reduced we would for a sound public health policy still be interested in securing construction material with the greatest negative potentialities.

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FURTHER OBSERVATION OF THE EFFECT OF LIGHT ON THE SYNTHESIS OF VITAMINS

By

V. G. HELLER AND RUTH REDER ST. JULIAN

(From the Department of Agricultural Chemistry Research, Oklahoma Agricultural and Mechanical College, Stillwater.)

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IN A previous article one of us (1) stated that vitamin A was present in greater amounts in wheat seedlings which had been exposed during the growing period to the open sunlight than in those grown in darkness; that the ultra violet, carbon arc, or the Mazda lamp, all caused an increase in the amount present as compared to the etiolated sprouts, but an amount less than that found in the sunlight-grown plants. The etiolated sprouts contained very little of the vitamin, if any. These findings compare favorably with those of other investigations mentioned in our previous article. Since that time Moore (2), after a carefully planned piece of work, states that seeds germinated and fed in complete darkness contain measurable, though small, amounts of this substance.

Vitamin B (B and G) was reported at that time as not increasing its amount during the germination period. While tests have not been carried out since the recognized division of B into two parts, in all probability had either increased it would have been noted in the previous tests. Vitamin C, we felt, increased somewhat more in seeds germinated in the light than in those kept in darkness. Since that time Matsuoka (3) has reported similar findings. However, Honeywell and Steenbock (4) and Eggleton and Harris (5) believe that germination alone is responsible for this result.

Chick and Roscoe (6) state that spinach contains little vitamin D. Boas (7) reports none present in winter-grown spinach. Stepp (8) states that germination produces no vitamin D, while Voltz and Kirsch (9) and Schittenhelm and Eisler (10) state that seedlings grown in the dark or in the sunlight possess antirachitic properties. While these reports are seemingly contradictory, they are somewhat parallel with our results reported in this article.

A number of interesting articles somewhat related to this problem have been reported. Dye, Medlock and Christ (11) state that the outer green leaves of lettuce are far superior to the colorless inner leaves in their vitamin A content. Steenbock and Sell (12) have stated the same for cabbage.

Christ and Dye (13) find similar results for green and uncolored asparagus shoots.

All these reports seem to indicate that vitamin A is in some way associated with the chlorophyll of the plant. However, other recent reports seem to contradict this assumption. House, Nelson, and Haber (14) and Morgan and Smith (15) find that green tomatoes contain less vitamin A than colored ones, regardless of whether they were ripened in the sunlight, in darkness, or artificially in an ethylene atmosphere. The latter authors state the same for red bell peppers.

Coward (16) reports that when leaves lose their green color and become yellow their vitamin content increases.

We have demonstrated (unpublished data) that yellow beans are as potent as related green varieties. It has often been suggested that the vitamin was associated with carotin, yet others have found potent sources quite devoid of this substance. These contradictions tend to prove that there is yet much to be learned in regard to the chemical composition of the vitamin. It is safe to postulate that the vitamin carrier is synthesized under conditions similar to the production of chlorophyll, but when the latter is transformed during the period of ripening, the vitamin retains its form. Considerable data in connection with this subject have accumulated since our last paper, and at this time we desire to report on certain aspects of the problem. The phases to be considered are: 1.—the effect of colored lights on the synthesis of vitamin A; 2.—the effect of blanching tissue which has previously become potent; 3.—the possible migration of the vitamin from one portion of the plant to another; and 4.—the effect of light upon the synthesis of vitamin D.

Experimental

The first series of tests are all concerned with vitamin A. A single brief description of the technique employed will serve for all the tests. The ration consisted of dextrin, 70.5 per cent; alcohol extracted and heated casein, 18 per cent; Fleischmann's dried yeast, 8 per cent; McCollum's No. 185 salt mixture, 3.5 per cent. The entire mixture was irradiated in thin layers prior to its use. The test animals were young rats from our own colonies weighing about 50 grams. The cages, which were of the usual type, contained four or more rats each, the animals having been selected to be comparable as to age, sex, and litter origin. In all cases the basic ration was fed until the growth curve of the animals became flat, at which time the supplements were fed directly to the animals in such a manner as to keep the food consumption of the various lots comparable.

The Effect of Blanching of Vegetables

Our previous tests having demonstrated that seedlings, etiolated by being kept continuously in the dark, were much less potent than seedlings raised in the sunlight, the next question suggested was whether a vegetable raised in the sunlight, then whitened by exclusion of sunlight, likewise loses its vitamin potencies. Wheat seedlings were raised in a washed sand medium until the green plants were about two inches in height. One tray was then placed in a dark room until practically etiolated, while the other tray remained in the sunlight. A third tray was kept through the entire time in darkness. Three cages of rats depleted of vitamin as previously explained were fed the seedlings. Lot 1 received 10 green sprouts per rat per day, lot 2 received an equal number of the seeds etiolated after growth, and lot 3 received a like number of seedlings germinated in complete darkness. The average of several such trials is shown in the following table. The first series was carried out in early winter and the second in early summer.

Table I

Type of Supplement	Days Fed	Average gain in weight
Sunlight-grown	35	25
Blanched	35	19
Etiolated	35	9
Sunlight-grown	70	51
Blanched	70	42
Etiolated	70	16
Green leaves of celery	55	76
White leaves of celery	55	67

These results indicate that once the vitamin has been synthesized it is not destroyed with the disappearance of the chlorophyll.

Such a question is of interest in the use of celery which has been etiolated after attaining full growth. A test of green and white outer leaves of celery was made in the same manner. The results are shown in Table I.

This is another indication that after once acquiring potency these leaves retain their vitamin. If some allowance be made for the increase of vitamin in the green seedlings which received the longer exposure, the results of the two seem to be practically equal.

Place of Formation or Storage of the Vitamin

An attempt has been made to determine whether the vitamin is equally distributed throughout the tissue of the germinating seeds. Wheat seed-

lings were grown in the sunlight in sand medium as before. When the sprouts were about two inches above the level of the sand they were used in the following tests. Rats depleted of vitamin A were divided into three cages. Lot 1 received 10 sprouts per rat per day, lot 2 received only the green tops of 10 sprouts per rat per day, and lot 3 received the seed remnant from which the tops had been removed. Table II records results of these tests.

TABLE II

Type of Supplement	Days Fed	Average gain in weight
Whole green sprouts	35	25
Tops	35	29
Seed remnants	35	9
Whole green sprouts	40	50
Tops	40	40
Seed remnants	40	10

The two groups of tests again represent the averages of lots, the first of which were carried out in cold weather and the second in early summer. These results indicate that the vitamin is formed and stored largely in the portion of the plant exposed to light and it evidently indicates that the vitamin does not migrate to the other portions of the plant. This would explain also why the inner white leaves of lettuce, or cabbage, are not so potent as the green outer leaves.

TO DETERMINE THE EFFECT OF COLORED LIGHTS OR LIGHT WAVES OF DIFFERENT LENGTH UPON THE SYNTHESIS OF VITAMINS IN GROWING PLANT TISSUE

In order to determine the comparative efficiency of long and short wave lengths upon the synthesis of vitamins, special glass filters were secured from the Corning glass works. The first was a blue glass (green glass G403ED) which transmitted waves of 435–490 μm, theoretically removing the greater portion of the long-wave red spectrum. The second was an infra red glass (infra red G554FF) transmitting only waves of 626–720 μm, removing the greater portion of the more visible spectrum. Wheat seedlings in sand media were grown under these filter screens in the open sunlight until the sprouts were approximately two weeks of age. Using the same technique as before, ten of the seedlings raised under the blue glass were fed to each of the rats in one cage each day. Ten seedlings per rat per day from under the infra red glass were fed to the second cage of rats

each day. The third cage received 10 seedlings per rat per day, raised in the open sunlight and were used as a control group. This experiment has been repeated ten times at various seasons of the year, and for various lengths of time. While the results are not always entirely comparable, probably due to the season of the year and the consequent variation in illumination, the general trend of results is the same. An average of the entire series representing over 40 rats in each case gives the following results.

Type of Supplement	Days Fed	Average gain in weight
Sunlight	46	53.
Green Glass	46	43.5
Red Glass	46	38.

An inspection of these data would indicate that the short wave was more important in this synthesis. Certain other factors need to be considered before drawing these conclusions too definitely. First, the seed bed and growing conditions remained more constant under the glass filters than in the open air, which should favor their potency. Second, the intensity of illumination must be altered. Either filter must reduce this effect. According to the manufacturer's investigation, the visible intensity is entirely removed under the infra red, yet we find the seedlings from this group only slightly less potent. Third, the short waves cannot be considered as entirely responsible for the changes, for again the infra red filter is supposed to screen out largely this end of the spectrum.

Evidently there is a series of factors involved, probably interrelated or affecting the others, namely, intensity of light, type of light wave, temperature, and possibly others. There is much to indicate that light waves beyond the spectrum are effective.

Effect of Light Upon Synthesis of Vitamin D

To test the effect of germination in light upon the formation of vitamin D, seeds were germinated as before, one lot in open sunlight, one under green glass, one under red glass, and the last in darkness. Five cages of rats, selected for the vitamin D work, were kept in a semi-darkened room and fed the Steenbock and Black (17) ration. When a rachitic condition was established by technique formerly outlined by us (18), the supplements were added. Cage 1 received 8 sunlight germinated seedlings per rat per day, cage 2 received an equal number of those grown under green glass, cage 3 received an equal number of those grown under red glass, cage 4 received an equal number of those grown in darkness, and the control lot

in cage 5 received only ungerminated seeds. Twelve days thereafter the skiagrams of all groups showed no cures. A second series of skiagrams taken 18 days after adding supplements showed those that had received seedlings from sunlight practically cured. No other series was completely cured, and after 24 days those receiving seedlings from under green glass were only partially cured, those under red glass showed no improvement, and those receiving etiolated seedlings and ungerminated seed showed a very critical condition. An examination of the data indicates that green leaves are not good sources of vitamin D, that seedlings grown in the open sunlight do synthesize vitamin D to a certain extent, and that no appreciable increase is noted in the absence of brilliant sunlight. Possibly we are dealing here with a mild irradiation rather than with synthesis, though an irradiation of dry seeds produced no parallel results. A parallel series of results with asparagus shoots, using as high as eight grams per rat per day, failed to produce a curative action in any case.

Another series of tests designed to compare the potencies of yellow and green beans, yellow carrots, and red beets failed to show any preferences, in fact, even when these substances made up fifty per cent of the ration, a cure was not effected in fifteen days. These tests tend to prove that vegetables cannot be depended upon as a source of the antirachitic factor.

Conclusions

1.—The results seem to indicate that after green leaves have become a potent source of vitamin through exposure to the sunshine, potency is not destroyed with the destruction of the color in blanching.

2.—As the vitamin forms, it seems to be stored in the portion of the plant most exposed to the sunlight and it is not transferred to the other portions of the plant, and the most actively growing portion, if etiolated, may be devoid of potency.

3.—It is evident that a number of interrelated factors are active in the synthesis in light. Neither wave length nor heat can be considered solely responsible.

4.—Wheat seedlings germinated in open sunlight possess some antirachitic properties; grown under green glass there is slight potency, while no beneficial results were obtained from seedlings grown under red glass or in darkness.

5.—Tests of green and yellow beans, carrots, and beets, failed to show any relationship between potency and color.

6.—Vegetables are a very poor source of vitamin D.

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THE MINERAL EXCHANGES OF MAN

I. ORGANIZATION OF METABOLISM WARD AND ANALYTICAL METHODS

By

SAMUEL H. BASSETT, C. A. ELDEN, AND W. S. MCCANN†

(From the Department of Medicine of the School of Medicine and Dentistry, University of Rochester, and the Medical Clinic of the Strong Memorial Hospital, Rochester, N. Y.)

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INTRODUCTION

UP TO the present time studies of the mineral exchanges of man, in the majority of instances, have been concerned with only two or three of the ash constituents. Many years ago Bunge (6) stated that the ingestion of an excessive amount of potassium caused a loss of sodium from the body. If this be true, then an interpretation of the behavior of any single mineral substance demands a knowledge of the simultaneous behavior of others, particularly of those which are known to be physiological antagonists, or of those which possess the power of displacing other ions from the body.

In the past two and one-half years we have made an effort to carry on more comprehensive observations of the mineral exchanges of man, observing the simultaneous behavior of sodium, potassium, calcium, magnesium, iron, nitrogen, and phosphorus, in the hope of discovering some of the fundamental relationships between these substances. The subjects studied have included normal men, patients with pernicious anemia before and during the blood regeneration induced by liver therapy, a patient with erythremia during blood destruction resulting from the administration of phenylhydrazine hydrochloride, certain patients with osteoporosis and degenerative arthritis, a patient with chronic jaundice of five years duration due to biliary cirrhosis, and an oedematous patient with chronic hemorrhagic nephritis during diuresis induced by the administration of potassium citrate. Further studies are in progress.

In order to carry out a program of study of the mineral exchanges of human subjects it is necessary to have a metabolism ward and diet kitchen especially adapted to this work. From the beginning, the Medical Clinic of the University of Rochester has possessed such a special metabolism unit in the Strong Memorial Hospital. One of us, W. S. M., having been

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trained by E. F. DuBois in the methods of the metabolism ward in Bellevue Hospital, copied the procedures used there as completely as possible. These have been described in a paper by Gephart and DuBois (8).

The metabolism unit consists of a well equipped kitchen flanked on each side by two rooms, each containing two beds. Between these rooms and the kitchen there are no doors, but glass partitions permit the nurses, as they work in the kitchen, to keep patients continually under observation. Each room has a small closet containing a commode chair in which is a weighed container for reception of excreta, for the use of patients who can get out of bed. For bed-ridden patients, a weighed bed pan is used. In the utility room adjoining, there are no facilities for the disposal of excreta so that it is impossible for specimen containers to be emptied by careless orderlies or cleaning women. Orderlies are forbidden to enter the unit except in company of a nurse. The personnel of the unit consists of a head nurse, a pupil nurse, a pupil dietition, a night nurse, and two chemical technicians. An assistant resident physician is assigned to this unit in addition to his other routine duties. The methods of weighing patients on a "silk" scale, of collecting urine specimens in separate bottles, of collecting feces, separating periods by carmine ingestion, weighing, and preparation of foods, are, in the main, identical with those described by Gephart and DuBois (8). The differences and exceptions will be noted in detail. Many helpful suggestions have been obtained from the paper by Bauer and Aub (5).

Collection and Preparation of Excreta

Each voiding of urine is kept separate in a tightly stoppered 20 oz. bottle, with a little toluene. The date, hour and minute of voiding are recorded in pencil on the etched area on the side of the bottle. It is stored in a refrigerator until ready to be sent to the laboratory. As the nurse receives each specimen, she records the fact on an invoice which is to be sent to the laboratory, noting the approximate volume of urine obtained by measuring the height of the urine on a scale. When the 24-hour urine is subsequently mixed and measured in the laboratory the loss of a specimen may be detected by the invoice and, at least, the approximate amount lost, is known. Mineral balances in this study have never been calculated on periods of less than five days. After measuring the mixed 24-hour specimen an aliquot part is saved and subsequently combined with the other daily aliquots corresponding to each period. Another portion is used for daily determinations of nitrogen and for such routine clinical examinations as are desired.

The moist weight, to the nearest gram, of each freshly passed stool is quickly obtained by weighing it directly in its container, the tare of which is known. It is then mixed to a paste with alcohol, and dried in the container on a steam bath. The dry weight of the stool is obtained by weighing it in its tared container before scraping it out. The portion of dry stool which adheres is small but is included in the calculation made from subsequent analysis of the portion removed. Weighings are made to 0.1 gram. The head nurse has the responsibility for separating stained from unstained fecal matter at the beginning of each period. In order to make this separation accurately the stool should be formed, hence cathartics should not be used. During the period bowel movements are secured if necessary by simple water enemas. Mineral oil is never used because it interferes with the preparation of the dried stool for analysis. The mixed specimens of dried stool are ground to a meal-like consistency with a porcelain pestle in a Coors mortar. The whole is saved in a tightly-stoppered container. From this an aliquot part of the whole for the period is removed and ground till it will pass a 20 mesh sieve, from which powder samples may be weighed for either dry or wet ashing. Occasionally a stool is encountered which contains a considerable amount of unabsorbed fat, so that it cannot be so finely ground. In this case, large samples are ashed to compensate for the slightly lesser accuracy of sampling.

The error involved in the changing water content of the dried stools, under the conditions of storage, must be considered. The maximum variation observed in one year amounted to about one per cent.

Some of the patients with pernicious anemia were quite ill in the early stages of the study. Such patients occasionally refused a portion of the food. It was at times impossible to weigh back rejected food in such a manner as to make an accurate subtraction of its mineral content from the calculated intake for the day. In such cases the rejected food was ground finely and added to the stools, without subtraction from the calculated intake. Vomitus was likewise added to stools, in the few cases in which emesis occurred.

The water balance of the body is intimately connected with its mineral exchanges. In nearly all cases a record of water balance was kept as follows: the *intake* was taken as the sum of all liquids plus an amount of water equal to the weight of the food *as served;* the *output* was the sum of all urine voided plus an amount of water equal to the weight of freshly passed stools, vomitus, and sputum, plus the water lost through the skin and lungs. An estimate of the latter item is obtained by correcting the differ-

ence between the other items of intake and output for the net change in weight during 24 hours. For example, a patient weighed 90.00 kg. at the start and 90.25 kg. at the end of a day, a net gain of 250 grams, ±10 gm. The total intake was 2580 cc. The total output through kidneys and gastro-intestinal tract was 1260 cc. The difference, 1320 cc., corrected by subtraction of 250 gm. water added to the body gives 1070 cc. as an approximation of the water lost through the skin and lungs. The errors in estimating water balance in this way are not large. Preformed water constitutes a large percentage of the ingesta. Of the solids of the ingesta, the indigestible residues excreted, will, in the long run exactly balance those ingested, and will correspond closely day by day when the diet is fairly uniform, or, if retained or lost in excess, will appear in the net change in weight of the subject. Of the digestible solids, foodstuffs of the ingesta, the portion unabsorbed will affect the balance precisely as the indigestible residues; the portion absorbed will either be deposited as such and corrected for by the change in weight or, if oxidized, will give rise to very nearly the same weight of water of combustion (16). Unless the complete energy balance be known it is impossible to calculate water of combustion more accurately, since the amounts of carbohydrate and fat burned cannot be determined. The calculation of the water balance is a most valuable check upon the accuracy of the nursing and dietetic procedures. It provides a means of doubly checking all operations, and of detecting errors.

In the preparation of foods the utmost care was taken to prevent losses. In general, the precautions described by Bauer and Aub (5) were taken. Foods cooked in water were prepared in individual servings, the water reduced to a minimum and special pains taken to secure ingestion of all the water which remained after cooking. The list of *foods* used in the study of mineral exchanges was carefully selected. The various items were chosen for relative constancy of composition and the availability of reliable analyses of mineral content. In some of the earlier experiments the ash constituents of each day's food were calculated from tables. A comprehensive experiment was carried out, which had the double purpose, first, of determining the accuracy of such calculations, and second, of working out menus of ten diets the mineral constituents of which were actually determined by analysis of the entire food for a day, ground, mixed, dried, sampled, ashed, and analyzed by exactly the same methods as were applied to the excreta (see below). The data obtained will be presented below. It may be said at this point that in the case of iron, magnesium, potassium, phosphorus, and nitrogen, the results agreed very closely with those

calculated from the tables of Sherman (17). In the case of calcium, the amounts found on analysis were consistently below the amounts calcu-

TABLE I

Diets	1 gms.	2 gms.	3 gms.	4 gms.	5 gms.	6 gms.	7 gms.	8 gms.	9 gms.	10 gms.
Apple		150		150	50					
Banana			30			40	150	100	40	100
Beef Rd.			30			100			40	
Beef tend'l'n.					100				120	
Bread	80	70	40	80	60	60	60	55	60	50
Butter	25	45	30	25	40	35	20	25	30	20
Cabbage	80									
Carrots		80		80						
Cauliflower					70					
Celery	20		20							20
Cheese	30	20		30		30	30	30		40
Cocoa								3		
Cod steak		90								
Cream, 4X	110		60	80	80	120	135	90	100	80
Egg	125	180	160	80	170	80	110	100	80	80
Farina			15			15		15		
Flour		5						8	3	
Grapefruit	100				100					
Grape juice		100								
Haddock							80			
Lamb chop	100									125
Lettuce	20	20	20	20	20	20	20	20	20	
Milk	120	200	190	280	230	300	170	430	340	260
Oatmeal	15			15					15	15
Onion		70	90			30			90	
Orange		80	200	80	60	150		100	150	100
Parsnip						70		70		
Pear				80						
Peas					80		80			
Pineapple							50			
Pork chop			120							
Potato	70	70	70	70	70	70	70	70	70	70
Prunes										20
Rice			10			20	20		10	
Shredded Wheat		20			20		20			
Spinach	80			70						70
Squash			70			70				
Strawberries	100				100					
Sugar	30	35	45	40	25	25	35	35	25	30
Tapioca		5					5			
Tomato	80	100	60	60	50	50	160	100	60	
Veal steak				120				90		

lated from the tables, and in the case of sodium, the amounts found were consistently above the amounts calculated; the calcium being 15.5 per cent below Sherman's figures, and the sodium 18.6 per cent above. The data given by Bauer and Aub (5) confirm our findings in the case of calcium. In Table I of their paper, their analyses of 18 items are compared with those of Sherman, the results of the analyses at the Massachusetts General Hospital being 12.8 per cent below those of Sherman, the average of a comparison of the whole 18 items.

Table I gives the composition of the ten diets used, expressed in terms of raw weights. It will be noted that a considerable variety of foods was used. Some of these are undoubtedly of variable composition, but the most variable items contribute a small percentage of the total ash constituents. Furthermore, it is apparent from the results in Table II, that, with

TABLE II

Diet No.	Cal.	P gm.	F gm.	C gm.	N gm.	P gm.	Na gm.	K gm.	Ca gm.	Mg gm.	Fe gm.
1	1984	64.2	126	134	10.3	1.076	1.565	2.620	0.781	0.257	0.0156
2	1959	60.9	96	199	9.7	1.294	1.585	2.427	0.664	0.203	0.0112
3	2003	67.4	116	158	10.8	1.070	1.003	2.483	0.471	0.178	0.0093
4	1998	66.4	108	176	10.6	1.223	1.728	2.670	0.839	0.247	0.0136
5	1976	64.0	117	155	10.3	1.140	1.213	2.253	0.566	0.200	0.0158
6	1979	65.0	110	168	10.5	1.254	1.350	2.590	0.809	0.198	0.0109
7	1996	65.4	104	185	10.5	1.194	1.416	2.580	0.701	0.249	0.0092
8	1978	64.6	114	159	10.3	1.224	1.342	2.540	0.936	0.200	0.0120
9	2011	68.9	121	147	11.0	1.069	1.082	2.440	0.612	0.206	0.0124
10	1986	63.5	121	146	10.2	1.195	1.305	2.760	0.848	0.202	0.0149
Total found					104.2	11.739	13.589	25.373	7.227	2.140	0.1249
Total calculated					111.2	11.939	11.464	26.365	8.557	2.155	0.1252
Per cent difference					−6.3	−1.7	+18.6	−3.8	−15.5	−0.7	−0.24
Per cent agreement of duplicate diets					±0.4	±0.1	±0.7	±1.7	±1.6	±1.0	±2.4

such a variety and with combinations of 5 or more diets in a period, the errors tend to balance each other.

Table II gives the composition of the ten diets compared with the mineral content as found by analysis. By comparison of the totals found and calculated for all ten diets one may estimate how much of a correction must be applied when a calculation is made from Sherman's tables (17) especially for calcium and sodium. It is quite possible that the geographical area from which the food supplies come will determine the magnitude of this difference. In view of this possibility it would be wise for workers in other regions to make similar tests. It is interesting to note in the same table the close percentage agreement between the analyses of two separate preparations of each diet, made in different years and at different seasons. The greatest variation was in the case of iron ± 2.4 per cent. In the later studies of mineral exchanges the intake for each period was calculated from the data in Table II. With ten diets analyzed it was possible to give a different diet on each day without having it fall monotonously on the same day of the week. All diets were calculated to 2000 calories. If it was desired to vary the total calories, this was done by an increment of some simple fraction, and each item on the menu was weighed out in the same proportion. That is if the menu for 2000 calories called for 100 grams of some item, for 2500 calories, 125 grams of this item would be weighed out, and a corresponding correction of the total ash constituents of the day's food would be made.

Analytical Methods

The methods of analysis employed were the following: nitrogen was determined by the Kjeldahl method; phosphorus by the method of Epperson (7); sodium by the method of Barber and Kolthoff (3, 4); potassium by the method of Shohl and Bennett (20); magnesium by the method of Epperson (7); calcium by the gasometric method of VanSlyke and Sendroy (22); and iron by the electrometric titration of King and Washburne (12). The complex nature of the material analyzed made it necessary to modify slightly some of the methods in order to obtain accurate results. Modifications, precautions as to technique, methods of ashing, etc., are cited below.

Ashing. Both dry and wet methods of ashing were employed in the preparation of samples for analysis. Of the two methods dry ashing gave the more uniformly reliable results. Large enough samples (20–30 gm.) could be ashed by this procedure to make errors due to sampling unlikely, and to provide enough material for all analyses. Moreover, the ash ob-

tained was more nearly completely soluble in dilute hydrochloric acid than that obtained by the use of the wet method. This greatly facilitated the preparation of the solutions from which the analyses were made. All diet samples were dry ashed, while many samples of urine and feces were ashed by the wet method. Both procedures are described.

Wet ashing was carried out in 800 cc. Kjeldahl flasks with a digestion mixture consisting of sulfuric and perchloric acids. Fifteen cubic centimeters of concentrated sulfuric acid (sp. gr. 1.8) and approximately the same amount of 60 per cent perchloric acid usually sufficed to ash 4 to 6 gms. of the dried material (feces or diet). The perchloric acid was added in quantities of 4 or 5 cc. at intervals during the digestion and the ashing considered complete when the sample had been reduced to a nearly colorless melt in the bottom of the flask. Considerable foaming occurred during the early stages of the digestion and careful regulation of the heat was required to prevent loss of material from the flask. In the case of urine, 1 liter was evaporated to a small volume in a large pyrex beaker on a steam bath, and quantitatively transferred to a Kjeldahl flask. Twenty to 30 cc. of concentrated sulfuric acid were added and the mixture heated cautiously over a free flame until fumes of sulfur trioxide were evolved. Perchloric acid was now added and digestion continued until ashing was complete. The ash and remaining acids were dissolved as completely as possible in about 150 cc. of dilute hydrochloric acid (1 to 10), filtered and the filtrate diluted with water to a volume of 200 cc. in a volumetric flask. When calcium was present in considerable amounts, calcium sulfate remained as a difficultly soluble residue. This together with any other residue remaining on the filter was fused with sodium carbonate and a separate solution of the fusion mixture prepared.

Dry ashing of stool and diet samples was carried out in 250 cc. porcelain crucibles placed in a large electric muffle furnace, the temperature of which was increased slowly to avoid active ignition and thereby decrease the possibilities of loss by spattering or convection by currents of gases. Volatilization of sodium and potassium salts was guarded against by keeping the maximum temperature of the furnace between 500 and 600° C. Ashing at this temperature is slow. At the end of 12 to 14 hours, the ash was digested with dilute hydrochloric acid (1–3) and any residue remaining removed by filtration through Munktells No. 1F ashless filter paper. The washed residue if it contained carbon was placed, together with the filter, in a porcelain crucible and treated with a few drops of nitric acid or superoxol (30 per cent hydrogen peroxide) and reashed. The extraction was re-

peated as before and the final residue which remained (usually very small in amount) after burning the filter was fused with sodium carbonate. The solution of the fusion mixture (as in the case of that prepared from wet ash) was stored separately from the main ash solution and used in connection with the latter only in determination of calcium, magnesium, and phosphorus.

Dry ashing of urine was accomplished as follows: 2 liters of urine in a large pyrex beaker were evaporated on a steam bath to a volume of 200 to 300 cc. treated with 50 cc. of concentrated nitric acid, the beaker covered, and the mixture allowed to digest for six to eight hours. The residue was now quantitatively transferred to a large porcelain casserole and as much water and nitric acid as possible removed by prolonged heating on the bath. Ashing was then begun by cautiously heating the casserole on a hot plate until most of the nitro-compounds had been decomposed and the residue carbonized. The casserole was finally transferred to the furnace, ashing completed and solutions prepared as described for stool and diet samples.

Nitrogen. Urine nitrogen was determined by the Folin-Wright simplified macro-Kjeldahl method (9). Nitrogen in diets and feces was determined in 1 to 2 gm. samples of the dry pulverized material by an official method (1).

Phosphorus and magnesium. The method of Epperson proved satisfactory for the determination of phosphorus and magnesium. After removal of calcium by precipitation with an excess of ammonium oxalate from a faintly acid solution (pH 4.8 to 5.2) (19) the filtrate was evaporated to dryness on a water bath and treated with an excess of concentrated nitric acid (10 to 15 cc.) to decompose ammonium salts (15). The nitric acid was removed and salts dehydrated as completely as possible by evaporation to dryness on the water bath. A small amount of insoluble silicic acid was usually formed at this point. The residue was taken up in a little dilute hydrochloric acid and any silica present removed by filtration through an asbestos Gooch. Five cc. of a 5 per cent solution of citric acid (15) were added with the magnesia mixture or phosphate solution to prevent the precipitation of iron and aluminium. A single precipitation of the magnesium ammonium phosphate gave sufficiently accurate results for the purpose of this study. Duplicate analyses agreed within 1 per cent. The use of filter paper was obviated in collection of the precipitate by filtering through weighed porcelain filter crucibles with fritted porcelain

bottoms.[1] Ignition of the crucibles for 1 hour in an electric furnace at about 800° C. was found sufficient to convert the magnesium ammonium phosphate into magnesium pyrophosphate.

Sodium. Phosphorus, and in the case of some of the stools which were wet ashed, potassium, interfered with the determination of sodium (3, 4). Phosphorus was readily removed by magnesia mixture[2] from samples of diet and urine ash. The small amount of sodium present in stool ash necessitated the use of relatively large samples for each determination. The high concentration of calcium in such samples resulted in the precipitation of most of the phosphorus as calcium phosphate. The amorphous character of this precipitate made its removal and washing difficult by the ordinary process of filtration. It was therefore removed by centrifuging in 100 cc. centrifuge tubes, the supernatant fluid decanted, and the precipitate washed at least 4 times in the same manner using 20 to 30 cc. portions of dilute ammonia for each washing.

Magnesia mixture was always added to insure complete precipitation of the phosphorus and the decanted fluid was allowed to stand 6 to 12 hours when any crystals of magnesium ammonium phosphate that had formed were removed by filtration. In all cases, before addition of the sodium reagent, the filtrate was evaporated to dryness and the residue dissolved in the least possible distilled water (usually 2 or 3 cc.). Samples taken from stool ash often contained a considerable quantity of ammonium salts. These were decomposed with nitric acid and evaporation continued to dryness.

Good results were obtained when samples of ash equivalent to 5 to 10 cc. of urine, 0.5 to 0.7 gms. of dried diet and 0.5 to 1.0 gm. of dried stool were used.

Potassium. The volumetric procedure described by Shohl and Bennett (20) for the determination of potassium as the iodoplatinate was used without alteration in these analyses.

Calcium. The gasometric method of VanSlyke and Sendroy (22) was slightly modified and used for the determination of calcium. Although a micromethod, designed for the determination of blood calcium, it was found applicable to the determination of calcium in urine, stool, and diet.

[1] May be obtained from Will Corporation, Rochester, N.Y.

[2] Precautions must be taken to obtain sodium free reagents. The C. P. magnesium chloride used in the laboratory was found to contain so much sodium that it could not be used in the preparation of magnesia mixture for use in the sodium determinations. A satisfactory magnesium salt was made by extracting 50 to 60 gms. of magnesium oxide 4 or 5 times with boiling water and dissolving it in pure hydrochloric acid.

In this laboratory it has consistently given more accurate results than either the volumetric procedure described by McCrudden (15) or that described by Shohl and Pedley (21).

Samples of ash were so diluted that quantities of 2 to 4 cc. contained from 0.2 to 0.6 mgs. of calcium. The portions for analysis were accurately pipetted into 15 cc. centrifuge tubes and one or two drops of 1 per cent alcoholic solution of methyl red added as an indicator. If not already distinctly acid the solution was acidified by adding a drop or two of hydrochloric acid and the volume brought to approximately 6 cc. with distilled water. One cubic centimeter of a saturated solution of ammonium oxalate was now added to each tube and the contents of the tube thoroughly mixed with a glass rod. Enough dilute ammonia (1 to 4) was added drop by drop with constant stirring to change the color of the indicator from red to a faint pink (intermediate between pink and yellow). The rod was removed and washed with a few drops of distilled water, the washings being permitted to fall into the tube. The tubes were placed in a rack and allowed to stand 6 to 12 hours. The remainder of the analysis was carried out exactly as described by VanSlyke and Sendroy (22).

Iron. Abundant references to methods for the determination of iron in biological materials appear in the literature. Of many methods tried that which gave the best results was the electrometric titration of King and Washburne (12). King and Howard (11) have used this method for the determination of iron in small quantities of blood, and in our hands it has proved adaptable to iron in urine, stools, and diets.

The principle involved is the familiar one of reduction of ferric iron to ferrous by titration with titanous sulphate or chloride, determining the end point electrometrically (13). If a sufficiently dilute solution of titanous sulphate is used for the titration, very small amounts of iron can be determined with accuracy. For details as to the assembling of apparatus, preparation, preservation, and standardization of the titanous sulphate, determination of the end point, etc., the reader is referred to the papers by King and Washburne and King and Howard. Following is a brief resumé of the principal variations from the original procedure.

It was found that the titanous solution could be stored in an atmosphere of pure carbon dioxide prepared in a Kipp generator and washed through a solution of acid mercuric chloride. Commercial carbon dioxide and hydrogen in cylinders are not sufficiently pure.

The E.M.F. set up between the platinum and calomel electrodes immersed in the solution being titrated was measured by means of a "Student

Type" potentiometer, with an enclosed lamp and scale galvanometer, both made by Leeds and Northrup. The platinum electrode was made from a 32 gauge bright platinum wire, fused to a copper wire of the same dimensions, which served as a connection in place of mercury. The platinum was fused into the end of a small glass tube. The titration flask was immersed partially in a water bath at a temperature of 85–90° C. during the titration, and was kept in motion by a motor shaker attached to its neck.

The quantity of iron in the sample to be analyzed should not be less than 0.25 mg. nor more than 3 mg. when an approximately 0.002 N solution of titanous sulphate is used for its reduction. Ten grams of the dried diets, or one gram of dried fecal material was sufficient for a single determination. The quantity of iron found in urine was extremely small, as a rule, in agreement with the findings of other investigators (18) (10) (14). It ranged from a trace to 0.7 mg. per liter. The ash of 400 cc. of urine was used for each determination.

Preparation of material for analysis of Iron. When feasible all specimens were ashed by the dry method, and dissolved in dilute hydrochloric acid as previously described under methods of ashing. Nitric acid was avoided in preparation of ash for reasons mentioned under the heading "Precautions." Aliquot parts of the ash solution were pipetted into 100 cc. pyrex beakers, 2 or 3 cc. of sulphuric acid (sp.g. 1.8) were added and the water and hydrochloric acid present removed by evaporation on a steam bath. The sulphuric acid and salts which remained were dissolved as completely as possible by warming with about 25 cc. of 20 per cent sulphuric acid and transferred to the titration flask. Enough 20 per cent sulphuric acid was used in washing the beaker to bring the volume of liquid in the flask to approximately 50 cc. In some instances considerable quantities of calcium sulphate precipitated on addition of the sulphuric acid. No attempt to remove this precipitate was made since it did not interfere with the iron determination. The 20 per cent sulphuric acid used as solvent was prepared as was that used in making the titanous sulphate solution and was stored in an atmosphere of carbon dioxide. The titration was performed as directed by King and Washburne (12).

Precautions were taken against the introduction of iron or other metals capable of being reduced, either through the use of impure reagents or through metal containers. Very small amounts of copper do not interfere, since iron may be determined in the presence of minute amounts of copper by this method (13). When oxidizing agents such as nitric or perchloric

acids were used in the ashing process it was necessary to remove all traces of these acids or their disintegration products prior to titration. To accomplish this the pyrex beakers were heated cautiously on a hot plate, after the water had been removed by evaporation on a steam bath. If the residue remaining in the beaker was small when fumes of sulphur trioxide ceased to be evolved, it was cooled and dissolved in 20 per cent sulphuric acid, and, if a considerable residue remained, 2 or 3 cc. more concentrated sulfuric acid were added and the process repeated to insure that all salts other than phosphates present in the ash had been converted into sulphates before transfer into the titration flask. Since interference by other ash constituents was never encountered it was never found necessary to separate iron from them.

Recovery experiments were made to determine the accuracy with which small amounts of iron could be recovered after addition to dried stool or diet specimens. When 2 mgm. of iron were added to a specimen of feces containing 6 mgm. all was recovered except 0.001 mgm., a negative error of 0.5 per cent. When 1 mg. was added to a dried diet sample containing 1.5 mgm. it was recovered with a negative error of 1 per cent. Routine use of the method has shown that the percentage error may be greater than that indicated by the recovery experiments, and that it may amount to as much as ± 5 per cent.

Summary and Conclusions

This paper gives in detail the technique for a comprehensive study of the simultaneous exchanges of sodium, potassium, calcium, magnesium, iron, nitrogen, and phosphorus.

1. The organization and methods of a special metabolism ward are described.

2. A comparison is made of the results of analyzing an entire diet for a day and of calculating the total mineral intake from tables. The values found for calcium were consistently lower than the calculated, and for sodium consistently higher. The discrepancies in the case of nitrogen, phosphorus, potassium, magnesium, and iron were of smaller magnitude, within the limits of accuracy of the procedure as a whole.

3. The same diet menus, weighed out and analyzed in different years, showed surprisingly close agreement on analysis.

4. The methods of preparation of foods and excreta for sampling, ashing, and analysis are given in detail.

The analytical methods used were selected after many trials as those giving the best results with the materials of both foods and excreta.

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THE RELATIVE VALUES OF THE PROTEINS OF LINSEED MEAL AND COTTONSEED MEAL IN THE NUTRITION OF GROWING RATS

By

W. W. BRAMAN*

(From the Division of Animal Nutrition, Department of Animal Husbandry, University of Illinois, Urbana.)

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IN THE interpretation of the results of investigations on rats concerned with biological measurements of the nutritive value of foods and food products, it is generally stated or implied that, at least relatively, the values secured are applicable to other species of animals and to man. There would be little defense for such investigations, aside from their contributions to comparative zoology, if this implication were not substantially true, and yet there is very little direct evidence on the point.

The recent paper of Bethke and his associates (1) concerned with the comparative nutritive value of the proteins of linseed meal and cottonseed meal for different animals directly challenges this viewpoint. The experimental data obtained have been interpreted to mean that for rats and calves the two proteins are of nearly equal nutritive value, but that for chickens cottonseed meal proteins are quite distinctly more efficient than linseed meal proteins, while for pigs the toxicity of cottonseed meal, fed at a level that proved non-toxic for the other species, prevented a comparison of protein values.

Because of the importance of the question involved, and because of the fact that the evidence adduced by the Ohio investigators did not in all cases relate directly and unequivocally to the protein values of their experimental rations, it seemed desirable to repeat the comparisons of the two feeds, using strictly quantitative and identical methods for each species of animal studied. The present paper reports the results of the experiments with albino rats.

THE PAIRED-FEEDING EXPERIMENT

The linseed and cottonseed meals used in all of the experiments were ground fine, partially dried in a low temperature oven, and extracted with ether. The cottonseed meal thus prepared contained 5.24 per cent of

* On leave of absence from the Institute of Animal Nutrition, Pennsylvania State College, State College.

moisture, 1.80 per cent of ether-soluble material, 10.94 per cent of crude fiber, and 7.09 per cent of nitrogen. The linseed meal contained 6.35 per cent of moisture, 0.27 per cent of fat, 10.09 per cent of fiber, and 6.31 per cent of nitrogen.

The meals were each embodied in a ration so as to furnish approximately 8 per cent of crude protein. The other constituents of the ration contributed minimal amounts of protein but adequate amounts of other known nutrients, so that each ration presumably was complete except for its protein content. The composition of the rations is given in Table I.

TABLE I
THE PERCENTAGE COMPOSITION OF THE EXPERIMENTAL RATIONS

Components	Cottonseed meal ration	Linseed meal ration
Ether extracted meal	18.05	20.29
Osborne and Mendel salts	4.00	4.00
Sucrose	10.00	10.00
Butterfat	7.72	8.00
Sodium chloride	1.00	1.00
Dried yeast	0.50	0.50
Cod liver oil	2.00	2.00
Starch	56.73	54.21
	100.00	100.00

The rations contained practically the same percentages of crude fiber and of fat. After drying at about 70° C. for 48 hours, they were analyzed. The cotton seed meal ration contained 1.349 per cent of nitrogen, and the linseed meal ration contained 1.394 per cent of nitrogen, equivalent respectively to 8.43 and 8.71 per cent of crude protein. In gross energy the rations contained 4435 and 4405 small calories per gram, respectively.

In the paired-feeding experiment, 8 pairs of rats were used. Each pair was of the same sex and from the same litter, and the pair mates did not differ in initial weight by more than 3 gms. The rats in each pair, one on the cottonseed ration and the other on the linseed ration, received the same amount of food, which was increased continuously until one or the other refused some of it. After reducing the amount offered until both rats cleaned it up, the daily food allowance was again increased. At the end of each week all rats were weighed, and all food residues were weighed and discarded. After the second week, each rat received daily two drops of tiki-tiki, prepared in the laboratory according to the directions of Wells (2). The feeding continued for 57 days.

The initial and final weights (averages of weights on three consecutive

days), the total gains, and the total food consumption of the rats are given in Table II. There is also included in this table the body lengths of the rats from tip of nose to anus, determined after killing the rats with ether.

In each of the 8 pairs of rats, the rat raised on the linseed meal ration exceeded its pair mate in total gain. Such a consistent outcome, favoring either one or the other ration, would have resulted from chance only, twice in 256 trials. The average of the 8 differences in total gain between pair mates is 8.4 gms., and their standard deviation is 3.3 gms. The average difference is more than 2.5 times the standard deviation. According to "Student's" probability tables (3), for n =8 and z =2.5 the odds are only 1 in about 10,000 that an average difference of this size or larger would have resulted by chance.

In Table III, a comparison of the weekly gains of pair mates is made. For each week and each pair of the experiment a sign is given to indicate which of the rats gained the faster, if their gains were different. Again the evidence is quite decisive that the linseed meal promoted a faster growth. In 41 comparisons the rat receiving the linseed ration gained the faster, in only 12 comparisons was the reverse true, while in 11 comparisons the gains of pair mates were equal.

TABLE II

SUMMARY OF BODY WEIGHTS, GAINS, FEED RECORDS, AND BODY LENGTHS

	Pair 1♂		Pair 2♂		Pair 3♂		Pair 4♀		Pair 5♂		Pair 6♂		Pair 7♀		Pair 8♀	
	Cotton-seed meal	Linseed meal	Cotton-seed meal	Linseed meal	Cotton-seed meal	Linseed meal	Cotton-seed meal	Linseed meal	Cotton-seed meal	Linseed meal	Cotton-seed meal	Linseed meal	Cotton-seed meal	Linseed meal	Cotton-seed meal	Linseed meal
Initial weight, gms.	54	52	47	47	52	51	51	54	63	61	49	51	52	54	49	49
Final weight, gms.	88	96	82	91	95	103	92	108	115	115	89	97	96	104	88	100
Gains, gms.	34	44	35	44	43	52	41	54	52	54	40	46	44	50	39	51
Total food, gms.	326	328	339	342	400	400	395	395	406	400	332	331	381	377	351	352
Body length, cms.	16.7	17.5	16.5	17.0	17.1	17.3	16.9	17.7	17.9	18.2	17.1	17.1	17.1	17.4	16.3	16.9

TABLE III

A COMPARISON OF THE WEEKLY GAINS MADE BY PAIR MATES

Week	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6	Pair 7	Pair 8	Totals per week +	−	±
1	+	±	±	±	±	+	±	+	3	0	5
2	+	+	+	+	±	+	+	+	7	0	1
3	±	±	−	+	+	−	+	+	4	2	2
4	+	+	+	±	−	+	+	+	6	1	1
5	−	+	−	−	+	±	+	+	4	3	1
6	+	−	+	±	−	+	+	+	5	2	1
7	+	+	+	+	−	−	−	+	5	3	0
8	+	+	+	+	+	+	+	−	7	1	0
	Totals per pair								Totals for experiment		
+	6	5	5	4	3	5	6	7	41		
−	1	1	2	1	3	2	1	1		12	
±	1	2	1	3	2	1	1	0			11

+ indicates a greater weekly gain by the rat receiving the linseed meal ration.
− indicates a greater weekly gain by the rat receiving the cottonseed meal ration.
± indicates equal weekly gains by pair mates.

For 61 weekly comparisons, excluding 3 whose significance was somewhat discounted by appreciable differences in the weekly consumption of food, the average difference in gain between pair mates was $.93 \pm .13$.

Referring again to Table II, it will be noted that the body length of the rats on the linseed meal ration exceeded that of the rats on the cottonseed meal ration in seven pairs, while in one pair (No. 6) the pair mates were of equal body length. The average difference was 4.4 mm. and the standard deviation of the 8 differences 2.6 mm., z thus being 1.7. The odds are only 1 in over 700 that as great or greater difference between pair mates would have been obtained by chance only. This evidence establishes the conclusion that the more rapid gains induced by the linseed meal ration over the cottonseed meal ration, when the amounts consumed were equalized, was due to a more rapid growth rather than to a more rapid fattening.

THE NITROGEN METABOLISM EXPERIMENT

The same two rations were used in a nitrogen balance experiment on 5 rats to determine the biological values of linseed meal and cottonseed meal proteins by Mitchell's modification (4) of Thomas' method. The rats were litter mates, 4 being females and 1 a male. Their initial age was about 7 weeks and their weights ranged from 51 to 61 grams.

The standardizing ration used in the initial and final periods had as its source of protein whole dried ether-extracted egg and contained 4.54 per cent of crude protein. It was made up of 5.02 per cent of the egg preparation, 4 per cent of Osborne and Mendel's salt mixture, 8 per cent of butterfat, 1.90 per cent of agar, 10 per cent of sucrose, 1 per cent of NaCl, 0.5 per cent of dried yeast, 2 per cent of cod liver oil, and 67.58 per cent of corn starch.

The collection periods were from 6 to 9 days in length, and were in each case preceded by at least 4 days of preliminary feeding on the same ration. In order to improve the consumption of the experimental rations, three drops of tiki-tiki were given to each rat daily after the first week, and in the final two periods the feed was mixed, in the individual feed cup, with hot water, instead of cold water, partially to dextrinize the starch. After the latter treatment there was less food refused by the rats. In the next to the last transition period, each rat was given daily 0.5 gm. of Northwestern Yeast Co.'s dried yeast up to within one day of the start of the following collection period, in a further attempt to stimulate food consumption.

The apparatus used was developed in Mitchell's laboratory for this type of work, and is the same as that already described in published articles except that the large crystallizing dishes containing the rats are covered, except for a small space around the edge, by large inverted glass funnels connected by rubber tubing to a suction pump. The pump affords effective ventilation for the metabolism dishes.

The results of the initial and final standardizing periods are given in Table IV. The purpose of these periods is to determine the excretion of metabolic nitrogen in the feces, per gram of food consumed, and of endogenous nitrogen in the urine, per 100 grams body weight. These factors differ in the two periods for each rat, and in computing the biological values for the two intervening periods, they are presumed to change in a linear fashion from the initial to the final value determined. In the computations of the intermediate periods, these factors are used in estimating the contribution of the body of the rat to the nitrogen appearing in the feces and in the urine.

In the second experimental period Rats 1, 2, and 3 received the cottonseed meal ration, and Rats 4 and 5 the linseed meal ration, while in the third period the rations were reversed for these two groups of rats. In Table V, the results have been assembled for the two rations.

The biological values obtained for the cottonseed meal proteins ranged from 75 to 80 and averaged 78. Those obtained for the linseed meal pro-

TABLE IV

THE RESULTS OF THE INITIAL AND FINAL STANDARDIZING PERIODS, EXPRESSED ON THE DAILY BASIS

Animal Number	Initial weight gms.	Final weight gms.	Average weight gms.	Food intake gms.	Fecal nitrogen mgms.	Body nitrogen in feces	Urinary nitrogen mgms.	Body nitrogen in urine
						Per gm. of food mgms.		Per 100 gms. weight mgms.
Initial period, dried egg protein ration, containing .727 per cent N								
1♀	50	52	51.3	5.2	7.73	1.49	19.00	37.04
2♂	54	55	55.0	4.6	8.35	1.82	15.82	28.76
3♀	53	55	54.3	5.5	9.71	1.77	22.68	41.77
4♀	52	54	53.5	5.2	8.79	1.69	19.60	36.64
5♀	57	63	60.3	5.8	8.38	1.44	16.60	27.53
Final period, dried egg protein ration, containing .727 per cent N								
1♀	78	81	79.8	6.5	17.42	2.68	27.54	34.51
2♂	78	83	80.5	6.5	14.07	2.16	21.74	27.01
3♀	82	85	83.8	6.5	13.07	2.01	26.20	31.26
4♀	82	85	83.8	6.5	12.93	1.99	26.34	31.43
5♀	91	95	93.0	6.5	14.21	2.19	28.44	30.58

TABLE V

THE BIOLOGICAL VALUES OF COTTONSEED MEAL AND LINSEED MEAL PROTEINS, RESULTS EXPRESSED ON THE DAILY BASIS

Animal Number	Initial wt. gms.	Final wt. gms.	Av. wt. gms.	Food intake gms.	N intake mgms.	Body N in feces: Fecal N mgms.	Body N in feces: Per gm. of food mgms.	Body N in feces: Per day mgms.	Food N in feces mgms.	Absorbed N mgms.	Urinary N mgms.	Body N in urine: Per 100 gms. wt. mgms.	Body N in urine: Per day mgms.	Food N in urine mgms.	Food N retained mgms.	Biol. value per cent
Cottonseed meal ration containing 1.349 per cent N																
1♀	59	65	62.0	5.94	80.13	26.99	1.89	11.23	15.76	64.37	36.28	36.20	22.44	13.84	50.53	78
2♂	64	67	65.8	5.45	73.52	25.98	1.93	10.52	15.46	58.06	32.78	28.18	18.54	14.24	43.82	75
3♀	60	66	63.3	6.20	83.64	27.64	1.85	11.47	16.17	67.47	39.56	38.27	24.22	15.34	52.13	77
4♀	78	82	80.3	6.50	87.69	31.34	1.89	12.29	19.05	68.64	40.34	33.17	26.64	13.70	54.94	80
5♀	85	91	88.3	6.50	87.69	28.29	1.94	12.61	15.68	72.01	40.90	29.56	26.10	14.80	57.21	79
															Average	78
Linseed meal ration containing 1.394 per cent N																
1♀	72	79	75.8	6.50	90.61	23.70	2.28	14.82	8.88	81.73	42.52	35.35	26.80	15.72	66.01	81
2♂	77	75	76.5	4.70	65.52	17.35	2.05	9.64	7.71	57.81	34.10	27.59	21.11	12.99	44.82	78
3♀	78	82	80.3	6.49	90.47	20.76	1.93	12.53	8.23	82.24	45.58	34.76	27.91	17.67	64.57	79
4♀	64	70	67.0	6.07	84.62	19.78	1.79	10.87	8.91	75.71	41.48	34.90	23.38	18.10	57.61	76
5♀	66	74	70.5	5.81	80.99	18.71	1.69	9.82	8.89	72.10	39.74	28.55	20.13	19.61	52.49	73
															Average	78

teins varied somewhat more, from 73 to 81, but averaged the same, *i.e.*, 78. In each case, apparently, an average of 78 per cent of the absorbed nitrogen of these meals was retained in the bodies of the growing rats, for the replacement of the endogenous losses of body nitrogen in maintenance and for the construction of new tissue in growth.

There is thus no difference in the utilization of the absorbed nitrogen of the two meals by growing rats, although in the paired-feeding experiment the linseed meal ration was distinctly superior in the promotion of growth. In searching the metabolism data of Table V for some clue to account for this conclusive superiority of the linseed meal ration in the promotion of growth, it appears that the protein (nitrogen) in this ration was digested to a distinctly better extent than the protein in the cottonseed meal ration. In Table VI, the computed coefficients of apparent and of true digestibility

TABLE VI

THE DIGESTIBILITY OF THE NITROGEN IN THE LINSEED MEAL AND COTTONSEED MEAL RATIONS

Rat No.	Linseed meal ration		Cottonseed meal ration	
	Apparent digestibility %	True digestibility %	Apparent digestibility %	True digestibility %
1	73.8	90.2	66.3	80.3
2	73.5	88.2	64.7	79.0
3	77.0	90.9	66.9	80.7
4	76.6	89.5	64.3	78.3
5	76.9	89.0	67.7	82.1
Average	75.6	89.6	66.0	80.1

of the nitrogen are summarized. The coefficients of apparent digestibility have been computed from the total fecal nitrogen excretion, while the coefficients of true digestibility have been computed from the estimates of absorbed nitrogen in Table V.

Apparently, on either basis, the linseed meal nitrogen was about 9 per cent more digestible than the cottonseed meal nitrogen, a difference that might account for the greater growth-promoting value of the linseed meal ration.

However, it is possible that the digestibility of the non-protein constituents of the two rations also varied. To test this possibility the two rations were fed in turn to four mature rats and the feces were collected, dried, weighed, and analyzed for nitrogen and for gross energy in the bomb

calorimeter. The collection periods were of 7 days duration, preceded by 4-day preliminary periods. The results of this test are summarized in Table VII. Here again, a distinct difference in the digestibility of nitrogen is evident, but the gross energy of the rations was about equally well utilized *i.e.*, to the extent of 91 to 92.

It may be concluded, therefore, that the superiority of the linseed meal ration in the promotion of growth was entirely due to the greater digestibility of its nitrogen.

The biological values obtained for cottonseed meal proteins and linseed meal proteins in these experiments, averaging 78 in each case, are higher than those reported by Bethke and his associates (1), *i.e.*, 72 and 71, respectively. This is probably due to the fact that in the Ohio experiments the proteins were fed at a higher level, 10 per cent as compared with 8 per cent.[1] Mitchell (5) has shown that the biological value of a protein decreases as its concentration in the experimental ration increases. Bethke and co-workers did not obtain the marked difference in digestibility of cottonseed meal and linseed meal nitrogen noted in these experiments. For the true digestibility of nitrogen in the cottonseed meal ration they obtained an average coefficient of 83.6, and for the linseed meal ration an average of 85.7.

Nevens (6) in this laboratory has reported the results of determinations of the biological value of cottonseed meal proteins for rats in rations containing 10 per cent of protein. Two trials were made on each of 3 rats. He obtained an average biological value of 66, and an average true digestibility of 74 per cent; the apparent digestibility averaged 61 per cent.

Summary and Conclusions

In paired-feeding experiments on white rats, a ration containing approximately 8 per cent of linseed meal proteins proved definitely superior in growth-promoting value to a ration similar in every respect except for the substitution of linseed meal protein for cottonseed meal protein. The former ration induced more rapid gains in body weight on the same amounts of food and more rapid growth in length of body. Eight pairs of rats were used in this test.

In nitrogen balance studies on five rats, the biological values of the

[1] It should also be noted that in these experiments the rations of all periods contained 6 per cent of yeast, supplying to the ration 2.5 to 3.0 per cent of protein above the 10 per cent furnished by the cottonseed and linseed meals. This constitutes in several respects a departure from the method used in the experiments under discussion, the effect of which on the biological values cannot be evaluated.

TABLE VII

THE UTILIZATION OF NITROGEN AND OF ENERGY IN THE LINSEED MEAL AND THE COTTON SEED MEAL RATION,*
RESULTS EXPRESSED ON THE WEEKLY BASIS

Rat	Body weight gms.	Food intake gms.	Nitrogen intake gms.	Energy intake cals.	Weight dry feces gms.	Nitrogen in feces %	Fecal nitrogen gms.	Digestibility of N %	Energy per gm. feces cals.	Fecal energy cals.	Digestibility of energy %
Linseed meal ration 1.394 per cent N 4405 cals. per gram.											
1♂	266	70	.9578	308	7.1990	3.70	.2664	72.7	4.002	28.8	90.6
2♂	285	70	"	"	6.8682	3.59	.2466	74.7	4.028	27.7	91.0
3♂	242	70	"	"	7.0264	3.60	.2530	74.1	4.173	29.3	90.5
4♂	297	70	"	"	6.6804	3.72	.2485	74.5	3.875	25.9	91.6
								Average 74.0			90.9
Cottonseed meal ration 1.349 per cent N 4435 cals. per gm.											
1♂	273	70	.9443	310	6.3686	4.87	.3102	67.1	3.988	25.4	91.8
2♂	288	70	"	"	6.3904	4.73	.3023	68.0	4.026	25.7	91.7
3♂	250	70	"	"	6.2501	4.87	.3044	67.8	4.106	25.7	91.7
4♂	297	70	"	"	6.3194	4.71	.2976	68.5	4.042	25.5	91.8
								Average 67.9			91.7

* Grateful acknowledgment is made to Mr. Frank Simpson, of the Division of Animal Nutrition, for performing this test of the experimental rations.

nitrogen in the two experimental rations were found to be the same, averaging 78 in each case. However, the digestibility of the cottonseed meal nitrogen was 9 per cent less than that of the linseed meal nitrogen. The gross energy of the two rations was shown to be equally digestible, so that the superior growth-promoting value of the linseed meal ration must have been due solely to its greater content of digestible protein.

It may be concluded that the biological values of linseed meal proteins and cottonseed meal proteins are the same for rats, but that their digestibilities may differ markedly, depending probably upon a variable inclusion of hulls in cottonseed meal.

Grateful acknowledgement is here made to Dr. H. H. Mitchell, head of the Division of Animal Nutrition, who proposed this study and supervised it throughout, giving kindly suggestions and criticisms.

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THE AGE FACTOR IN THE RESPONSE OF THE RAT TO LEVEL OF DIETARY PROTEIN*

By

ARTHUR H. SMITH AND T. S. MOISE

(*From the Laboratory of Physiological Chemistry and the Department of Surgery, Yale University, New Haven.*)

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DURING the past decade much has been written about the deleterious effect of diets rich in protein. Since the kidney is the organ chiefly concerned with the removal of nitrogenous products in the metabolism of protein, attention has naturally been focused on this organ. However, in view of the apparent simplicity of the thesis, the experimental results have been surprisingly variable. It seems, therefore, that any controlled observations of the effects of high concentrations of dietary protein will be valuable in helping to clarify a situation which involves a subject of considerable practical importance.

Among other confusing statements in the literature are those concerning growth and physiological well-being of experimental animals on rations rich in protein. In some cases poor growth is reported; in others, satisfactory early growth but failure to reach the usual mature body weight; while normal development is observed in still other studies. In view of the lack of concordance in this respect and in the light of recent accounts (1, 2, 3) of rapid growth of albino rats—the animal most frequently used in these experiments—it seemed desirable to examine anew the influence of dietary protein upon the development of individuals of the same strain of rapidly-growing animals. The increase in body weight in young animals and maintenance in old ones should be a reliable criterion of the adequacy of a diet under conditions where other variables are ruled out. In the present report we have attempted to correlate change in body weight with concentration of dietary protein in white rats of different ages.

The observations were made upon a large number of male albino rats from all of which the right kidney had been removed. They were grouped into four different ages and were given complete diets containing three different amounts of protein (see Table I). At 56 days and 150 days after nephrectomy (the experimental ration was first given on the day of the

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operation) rats from each age group were killed and observations and organ measurements made. The data were used only from those animals which at

TABLE I

COMPOSITION OF EXPERIMENTAL RATIONS

Food material	Diet 18	Diet 60	Diet 85	
Casein	18%	60%	85%	700 mgm. dried yeast daily
Hydrogenated veg. fat*.	22	22	8	given apart from basal ration.
Cod liver oil	5	5	4	Casein contained 12.1% N,
Corn starch	51	9		8.2% moisture, 5.2% ash.
Salts	4	4	3	For preparation of salt mix-
Energy value per gm.	5.2 Cal.	5 Cal.	4.2 Cal.	ture see Osborne, T. B. and
Protein calories	12%	38%	67%	Mendel, L. B., *Jour. Biol. Chem.*, 1917, XXXII, 374.

* Crisco.

autopsy had no evidence of pulmonary infection, and are summarized in Table II.

It is seen that all the animals which were 30 days old at the beginning of the experiment showed very satisfactory growth both for 56 days and for 150 days. It has previously been shown (4, 5) that unilateral nephrectomy *per se* is attended with no demonstrable alteration in rate of growth when the diet contains fairly low concentrations of protein. In the present study, however, even on the ration containing moderately high concentration of protein (Diet 60) the growth over a period of 56 days compares favorably with the rapid growth records recorded in the recent literature (1, 2, 3). Within this age group (30 days), however, it is obvious that the gain in weight over 56 days and over 150 days period is distinctly less on the very high-protein ration than on the other diets. Like observations were made by Smith and Jones (4) on young rats given similar rations for a much longer time. It is interesting to note that the ratio of total calories ingested to grams of gain is almost indentical on all three diets. The favorable responses of the group on Diet 60 and the group on Diet 85 over the period of most rapid growth, together with the poorer behavior of older animals on Diet 85, seem to confirm the principle emphasized by Burr and Burr (6) that the nutritive ratio should be relatively high during rapid growth and lower as it proceeds beyond this period.

In the group 90 days old at the beginning of the experiment there is naturally less increase in body weight, but the relative gains on the Diet 60

TABLE II

Ration No.	Age at beginning	Duration	Number of rats	Average body wt. at beginning	Average final body wt.	Per Cent change in body wt.	Total calories consumed	Total Calories per gm. gain in body wt.	Average gain per day
	days	days		grams	grams				grams
18	30	56	9	77	282	273± 60.2	2841	13.9	3.7
60	30	56	8	80	347	333± 47.9	3253	12.2	4.8
85	30	56	10	74	249	238± 53.5	2404	13.7	3.1
18	30	150	12	65	468	619± 90.6	9416	23.4	2.7
60	30	150	11	68	413	519± 95.4	8105	23.5	2.3
85	30	150	12	77	403	445±133.8	7899	24.2	2.2
18	90	56	14	274	408	53± 16.5	3593		
60	90	56	12	228	347	53± 9.5	2915		
85	90	56	14	280	351	22± 4.6	2822		
18	90	150	6	253	441	74± 12.1	8736		
60	90	150	8	251	461	85± 16.6	9445		
85	90	150	9	293	384	30± 7.1	7480		
18	180	56	14	431	460	5± 7.0	3240		
60	180	56	12	355	414	16± 7.6	3060		
85	180	56	16	396	390	−1± 5.0	2835		
18	180	150	17	399	492	27± 17.6	8674		
60	180	150	11	401	457	14± 8.0	7715		
85	180	150	16	378	394	4± 6.8	7640		
18	360	56	8	513	536	5± 4.7	3645		
60	360	56	3	435	455	5± 4.7	3405		
85	360	56	11	516	446	−13± 8.6	2944		
18	360	150	8	491	537	8± 13.9	9651		
60	360	150	8	442	494	6± 5.9	9455		
85	360	150	12	480	435	−10± 6.8	7724		
				LAPAROTOMY					
18	90	56	5	274	418	53± 8.3	3650		
60	90	56	6	251	370	49± 11.1	2730		
85	90	56	4	307	404	32± 8.0	3389		
18	90	150	7	263	465	84± 35.9	9625		
60	90	150	4	252	410	72± 12.0	7580		
85	90	150	6	274	336	35± 4.7	7686		

equal or exceed those on the low protein ration. While the animals to which Diet 85 was given did not grow so well as those on the other two rations, they were in excellent physical condition at autopsy. In a small group of operative control rats 90 days old, the relative change in body weights of the groups on the three diets is similar to the animals with one kidney and again shows the favorable effect of a ration moderately rich in protein, as well as the retarding influence of very high concentrations of dietary protein.

As might be expected, the data for the rats 180 days old show smaller relative gains in weight. Here again the diet containing the very high concentration of protein permits the least gain though the animals in this group were in good condition after 150 days.

The rats 360 days old at the beginning of the experiment gained little. Even in this group Diet 60 compares favorably with the low protein ration after 150 days. The animals on Diet 85 lost weight and showed noticeably less subcutaneous and intra-abdominal fat at autopsy than did those on Diets 18 or 60. The data presented by Jackson and Moore (7) show essentially the same unfavorable change in body weight of their older rats with one kidney, compared to younger animals similarly treated, when all were given a diet relatively rich in protein over periods varying from 2 to 15 months. The largest individual final body weight in the group on Diet 85 was Rat 834 which weighed 496 grams after losing 30 grams in the course of 150 days; on Diet 18, Rat 1011 which weighed 703 grams after 150 days; and on Diet 60, Rat 1097 weighing 600 grams after 150 days.

The energy intake of the rats with one kidney is less on the very high protein diet than on the other rations in all the age groups, which fact would explain the failure to make the expected gains in body weight. There seems little room for doubt that this decreased food consumption is associated with some circumstance of the metabolism of the large quantities of protein ingested. The fact that the blood urea concentrations are markedly higher in the rats with one kidney on Diet 85 than on the other rations appears to substantiate this conclusion, although urea itself might not be the causative agent, for it has been shown that enlargement of the kidneys and renal damage resulting from feeding rations rich in protein do not occur when an equivalent amount of excess nitrogen is given as urea (8). That this is probably not the only explanation is suggested by the operative control group in which the animals on Diet 85 consumed more energy than the rats on Diet 60, yet made less gain in weight. However, the numbers of animals are too small for definite conclusions to be drawn in these latter

groups. That the rations were adequate from the qualitative point of view was shown by complete absence of symptoms or lesions attributed to a deficiency of accessory food factors or salts.

On the basis of the data collected in the present study it appears that under the conditions imposed by unilateral nephrectomy, maintenance, growth and general well-being are promoted in rats of widely different ages when the ration contains 38 per cent of the calories as protein, as well as or better than when only 12 per cent are derived from this foodstuff. Furthermore, good, though not maximal, rates of growth are supported in young rats 30 days of age at the beginning of the feeding period on a ration, 67 per cent of the total calories of which arises from its protein content. This ration, however, is not favorable for maintenance or growth of rats 6 and 12 months old over periods of 56 or 150 days.

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FAT SOLUBLE VITAMINS

XXXII. THE DISTRIBUTION OF VITAMIN A IN TOMATO AND THE STABILITY OF ADDED VITAMIN D*

By

H. STEENBOCK AND INEZ M. SCHRADER†

With the Coöperation of Blanche M. Riising and Alice M. Wirick

(From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.)

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THERE are two elements of interest in modern studies on the occurrence and distribution of vitamins. One is founded on the intensely practical objective of rationalizing the process of compounding a satisfactory ration. The other is founded on the desire to learn more about the nature of vitamins and to elucidate the mysteries of their function in the metabolism of both plants and animals. These combined prospects should certainly furnish enough inducement for the careful study of the vitamins of food materials especially those which are particularly rich in one or more of them. In this paper, we have chosen to give consideration to the vitamins of tomatoes.

HISTORICAL

The vitamin content of tomatoes has attracted attention ever since Givens and McCluggage (1) and Hess and Unger (2) in 1918 pointed out that tomato is a potent source of the antiscorbutic vitamin. The former found that 10 gms. of fresh raw tomato daily protected guinea pigs from scurvy, and when scurvy was already incident this amount cured the disease. They found that even dried tomatoes prepared at 55 to 60° with an exposure of 14 to 24 hours contained some vitamin C. Hess and Unger secured protection of guinea pigs from scurvy with 5 cc. of tomatoes which had been stored in a canned condition for almost a year. They thereupon fed canned tomatoes in amounts of 7.5 to 15 cc. to infants with good results. This led them to recommend canned tomato in place of the more expensive orange juice as an antiscorbutic.

In 1920 Hess (3) recommended canned tomato unreservedly for infant feeding in daily doses of one ounce. Hess and Unger (4, 5) reported that 5 cc. of strained canned tomato per day were necessary to protect guinea pigs from scurvy; 3 cc. were found insufficient. In view of the fact that vitamin C is generally less stable under alkaline conditions, they made tomato juice alkaline

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just before feeding, but observed no decreased potency. Boiled canned tomato juice was found somewhat less efficient.

Remy (6) found tomatoes sterilized by heat to contain considerable amounts of vitamin C, even after storage from 1 to 7 months. Givens and McCluggage (7, 8) found that a daily supplement of 2.5 gms. or even 1 gm. of fresh raw tomatoes would protect a guinea pig from scurvy. Heat processed tomatoes varied in their protective action. Tomatoes canned by the usual method of processing with 5 pounds steam pressure for 10 minutes or even 15 pounds for 30 minutes in daily doses of 10 gms. prevented scurvy in guinea pigs. Tomatoes dried at 35–40° C. for 52 hours were protective when fed in daily doses of .5 gm. La Mer, Campbell, and Sherman (9) studied the time curve of destruction of vitamin C by boiling filtered canned tomato juice. At the natural acidity of pH 4.2, one hour of boiling destroyed 50 per cent of the vitamin; at a reduced acidity of 5.1 the destruction increased to 58 per cent, and with a further reduction of acidity to a pH of 8.3 to 10.3, one hour of boiling destroyed 61 to 65 per cent. Three cc. of untreated canned tomato were found to give complete protection to a guinea pig.

House, Nelson, and Haber (10) found green tomatoes relatively poor in vitamin C. Air ripened and ethylene ripened tomatoes were richer in this vitamin than the green fruit, and vine ripened tomatoes were found superior to either the artificially ripened or the green tomatoes. The commercial method of ripening tomatoes in an ethylene air mixture was found to produce fruit equally as rich in vitamin C as fruit picked green and ripened in the air. Jones and Nelson (11) found the vitamin C content of tomatoes to increase as the fruit developed into a mature ripened condition. Naturally ripened tomatoes contained the most vitamin C. Ethylene ripened tomatoes contained no more vitamin C than the green fruit from which they had been prepared.

The latest report on the vitamin C content of tomatoes relative to its production and destruction is that by Clow and Marlatt (12). They found that there was an increase in vitamin C content with maturation of the fruit. Greenhouse tomatoes were found somewhat inferior but both greenhouse and field tomatoes picked green and then allowed to ripen at room temperature proved to be as potent as those ripened on the vine. Ripening with ethylene did not appear to prevent the usual development of vitamin C. Ripe tomatoes canned by the cold pack method were found as rich in vitamin C, nine months after storage, as freshly picked ripe tomatoes. However, after 15 to 20 months storage there were indications of some destruction. Green pickled tomatoes contained but little vitamin C. Of field ripened tomatoes 3 cc. daily were found sufficient to cure scurvy in a guinea pig.

That tomatoes contain vitamin B has also been known for some time. Hess and Unger (13) in 1919 found that pigeons could be cured of polyneuritis by the administration of 5 cc. canned tomato daily. Osborne and Mendel (14) in the same year included tomatoes in the list of fruits and vegetables in which vitamin B had been found to be present by experimental trial with rats. In 1920 Osborne and Mendel (15) found canned tomatoes dried at 60–70° C. rich in water-soluble vitamin B. One gm. daily doses promoted good growth. Five tenths gm. was not so effective, but even .2 gm. occasionally promoted limited growth. Osborne (16) has stated that tomatoes are among the richest of the vegetables on the dry basis in vitamin B, with spinach a close second.

Sherman and Burton (17) found that vitamin B in filtered tomato juice was more easily destroyed if made alkaline or even when the acidity was merely slightly decreased. The increased destruction upon heating for one hour at 100° at a pH of 7.9 instead of the natural pH of 4.28 was 20 to 30 per cent; at 9.2, 60 to 70 per cent; and at 10.9, 90 to 100 per cent. Oxygen did not appear to play a prominent part in the destruction. House, Nelson, and Haber (10) found that green tomatoes compared with ripe tomatoes were equally rich in vitamin B, and that ethylene treatment had no effect. It should, however, be mentioned that they observed some superiority in the ripened fruit, which was not regarded as of special significance. Jones and Nelson (11) found naturally ripened tomatoes a better source of vitamin B than any others, whether treated or untreated with ethylene. In a second series of experiments they obtained lower values for vitamin B

with the same fruit which had in the meantime been kept in storage. It suggested to them a partial destruction of the vitamin B with storage, but no definite conclusions were drawn.

That cod liver oil (18) butter fat (19, 20) and the green leafy parts of plants (21) are rich in vitamin A, and that this vitamin occurs abundantly in nature associated with yellow pigments (22, 23) of the carotinoid type has been known for some time. As a matter of fact, Euler and Hellström (24) found .005 mg. carotin to produce growth in rats when they were provided with vitamin D. Lycopin, the red isomer of carotin in tomato, was found inactive. Duliere, Morton and Drummond (25) however, found carotin inactive when highly purified. Moore (26) in turn has criticized the work of the later, and supported v. Euler and co-worker in their claims.

Whatever the facts may be in regard to carotin as to its serving in the capacity of vitamin A, or even its identity with vitamin A, carotin has long been known to be present in tomatoes (27); and tomatoes were found rich in vitamin A as early as 1920. At that time, Osborne and Mendel (28) found .1 gm. of dried tomatoes efficacious in supplying sufficient vitamin A to rats (29). Sherman and Munsell (30) found that .11 gm. of tomato daily, reinitiated growth in rats on a vitamin A-free diet. Of this tomato .17 gm. contained 1 rat unit of vitamin A as defined by them. Davis and Stillman (31) after carrying out experiments on rats suggested that tomatoes, among other fruits, could be used instead of orange juice to supply vitamin A to infants. Kohman (32) reported tomatoes 40 times as rich in vitamin A as strawberries. Sherman, Quinn, Day, and Miller (33) studied the stability to heat of vitamin A in tomato juice filtered free from suspended material in the same manner in which they studied the stability of vitamin B in this medium. When heated for 4 hours at 97°, 17 per cent of the vitamin A was found destroyed. An oxygen-free atmosphere did not reduce the destruction, nor did bubbling of air through the juice increase it. Changing the pH from 4.2 to 9.2 did not increase the destruction by heating in an atmosphere of nitrogen. Morgan and Smith (34) discovered the relative poverty of green tomatoes as compared with ripe tomatoes in vitamin A and its consistent development with the process of ripening. They pointed out that this was unexpected in view of the claims of Dye, Medlock, and Crist (35) that vitamin A and chlorophyll are produced simultaneously. They rather observed a simultaneous development of lycopin and vitamin A. Vine-ripened tomatoes and those ripened off the vines under various light conditions appeared to be equally rich in vitamin A. The adequacy of the ethylene ripening process was indicated. House, Nelson, and Haber (10) by feeding rats 2 gms. of tomatoes daily found green tomatoes lower in vitamin A content than ripe ones. With the latter the method of ripening did not appear to be a factor. Jones and Nelson (11) found naturally ripened tomatoes a better source of A than any other studied, including those ripened in ethylene. Green tomatoes did not vary in vitamin A content with stage of development or with ethylene treatment.

From the preceding review of the literature it can be appreciated that tomato is a potent source of vitamins A, B, and C. Hess (3) in 1920 when discussing tomato as a source of vitamin C, made the following statement, "As they have been shown by Osborne and Mendel to be rich in the water-soluble and the fat-soluble vitamins, canned tomatoes may be regarded from a nutritional standpoint as a palatable solution of the three vitamins." Morgan and Field (36) however, have reported that apricots compare favorably with the best figures reported for spinach, egg yolk, or butter. Peaches and prunes had less vitamin A than the apricots but as much or more than tomatoes, bananas, or lettuce. In our experiments we have found tomatoes from 1/5 to 1/10 as rich in vitamin A as a sample of good June butter.

Experimental

Vitamin A

In 1921, during the course of our studies on the relation of pigment to vitamin A, we had occasion to test tomatoes for their vitamin A content.

Fresh tomatoes were dried at a temperature approximating 100° until they were friable. They were then ground to a powder and incorporated in a basal ration consisting of casein, yeast, salt, agar, and dextrin or white corn, which constitutes a ration suitable for testing for the presence of vitamin A with rats. The dried tomato was mixed into these rations at a level of 2 and 5 per cent. To our surprise we found that even 2 per cent of dried tomato furnished enough vitamin A to allow young rats to grow in 16 weeks from a weight of 70 gms. to 290 gms. without any symptoms of ophthalmia or any other signs of vitamin A deficiency. Some of the females, as a matter of fact, produced a number of litters in the course of 18 weeks. This demonstrated to us an unexpected stability of vitamin A to the drying process, because the dried tomato, as a matter of fact, no longer resembled fresh material as to color; it had carmelized so that the final preparation was almost black.

It has been the object of our present experiments to determine how small an amount of tomato would suffice to furnish the rat with a sufficiency of vitamin A, and furthermore, to find out something about its distribution in this fruit. This problem has commercial as well as scientific significance, because there are now appearing on the market a number of tomato preparations for use as beverages. In some of these the entire tomato is macerated, freed from skins and seeds, and bottled as a permanent colloidal suspension of finely divided pulp. In other preparations, the tomato serum is separated from the pulp, leaving a pale yellow, perfectly clear solution. We have determined the distribution of vitamin A in a high grade commercial preparation.[1] Though sterilized by heat in the usual commercial manner, it contained sufficient vitamin C to cure a guinea pig of scurvy when given in amounts of 4 cc. daily. It was, therefore, evident that in its preparation it had not been unduly exposed to heat or oxygen.

Seventy-five white and piebald rats were used for our experiments. They were taken from stock at approximately 50 gms. in weight at 3 weeks of age and placed on screens in groups of 5 to 7. They were fed a basal synthetic ration free from vitamin A consisting of alcohol-extracted and heated casein 18, salt No. 40, 4, (37) agar 2, yeast 8, dextrin 68. The entire ration was treated with ultra-violet light from a Cooper Hewitt quartz mercury vapor lamp to supply it with vitamin D. Two hundred and eighty gram portions were spread out in a very thin layer in galvanized iron pans two feet square, and exposed for 30 minutes at a distance of 18 inches from

[1] The tomato used in these experiments was furnished by the Kemp Bros. Packing Co.

the burner. After 15 minutes exposure the material was stirred once. The lamp used was of a BY type which was run at 50 to 60 volts with a current density of 4 to 5 amperes.

The animals were fed as much of the ration as they cared to consume. No consumption records were kept. Symptoms of ophthalmia appeared at about the fourth or fifth week, but occasionally they appeared between the third and fourth weeks. With incidence of ophthalmia decline in weight usually resulted. When the ophthalmia gave every indication of being of a permanent rather than a transitory type, and when the eyes were severely erythemic but not purulent, the rats were taken for test. They were taken out of the different groups and segregated individually in small cages provided with screens. They were then given small amounts of the tomato preparation in addition to the basal ration. These were measured out by means of pipettes into small white glass dishes. The tomato was always consumed readily. The animals were observed daily and from week to week they were weighed and notes taken on the progress of their ophthalmic condition. In certain instances notes were taken daily, but this was the exception rather than the rule.

Some of the animals developed infections of the respiratory tract even before they were changed to the supplemented diet, and others developed this condition after the tomato feeding was started. Fortunately, these animals were few in number, and in most cases died shortly after the experiment was started so that the results were but little influenced by them. During the early course of the experiment, an attempt was made to distribute the members of the litters in such a way that they were apportioned equally among the different experimental lots. Some attention was also given to the equal distribution of the sexes; but later, when it was found that neither of these two factors was important, they were no longer given any consideration.

In order to conserve space the results of treating ophthalmia with tomato serum and whole tomato are presented in condensed form in Tables I and II and Chart 1. As seen in the tables most of the rats were started in what we designated progressive stage 3 of ophthalmia. This is marked by a persistent erythema, accompanied by swelling, sometimes with exudate and bareness of the lids, but with complete absence of purulency and the eyes either open or closed. This stage of ophthalmia is fairly definitely characterized and of a sufficient degree of severity to indicate slight improvement with treatment. On the other hand, when left untreated, it goes rapidly into the purulent stage, which we have designated as stage 4.

TABLE I

CURE OF OPHTHALMIA ON WHOLE TOMATO

Lot No.	Whole tomato daily cc.	Rat No.	Condition of ophthalmia when put on tomato	Condition of Ophthalmia Week by Week											
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
25	1/8	67	2	2	1	1	1	1	1	1	1	died			
		61	4	4	4	3	3	3	died						
		64	3	2	2	2	1	1	1	1	died				
		73	4	4	3	3	2	2	2	1	died				
18	1/4	31	3	4	3	2	2	2	2	2	1	1	cured		
		32	3	2	2	1	1	1	1	1	1	1	cured		
		33	3	3	2	2	2	2	1	1	1	1	cured		
		34	3	2	2	1	1	.1	1	1	1	1	cured		
19	1/2	48	4	3	2	2	1	1	1	1	cured				
		50	3	2	2	2	2	1	1	cured					
		51	2	2	1	1	1	1	1	cured					
		52	3	2	2	2	1	1	1	cured					

Key to Ophthalmia Tables

4. Purulency accompanied by closed eyes with severe swelling and occasional blindness.
3. Absence of purulency, but persistence of erythema bareness, swelling and exudate with eyes open or closed.
2. Absence of erythema, but eyes still swollen, cornea cloudy, presence of exudate eyelids bare.
1. Absence of acute reaction, but eyes slightly dirty or watery with or without protrusion of cornea and presence of scar tissue.
0. Normalcy, absence of exudate, bareness of lids and scar tissue.

TABLE II

CURE OF OPHTHALMIA ON TOMATO SERUM

Lot No.	Tomato serum daily cc.	Rat No.	Condition of ophthalmia when put on tomato	Condition of ophthalmia week by week											
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
10	1	5	3	4	4	4	4	died							
		13	3	2	died										
		17	3	4	4	4	died								
		25	3	3	2	2	died								
11	2	9	2	2	1	1	1	1	1						
		1	3	4	4	4	4	4	died						
		18	3	3	3	2	2	1	1						
		28	3	3	2	2	2	2	2						
16	4	58	2	2	1	1	1	1	1	1	1	1	1	died	
		8	3	3	2	2	2	1	1	1	1	1	1	died	
		14	3	3	2	2	2	2	2	1	died				
		24	2	3	2	3	died								

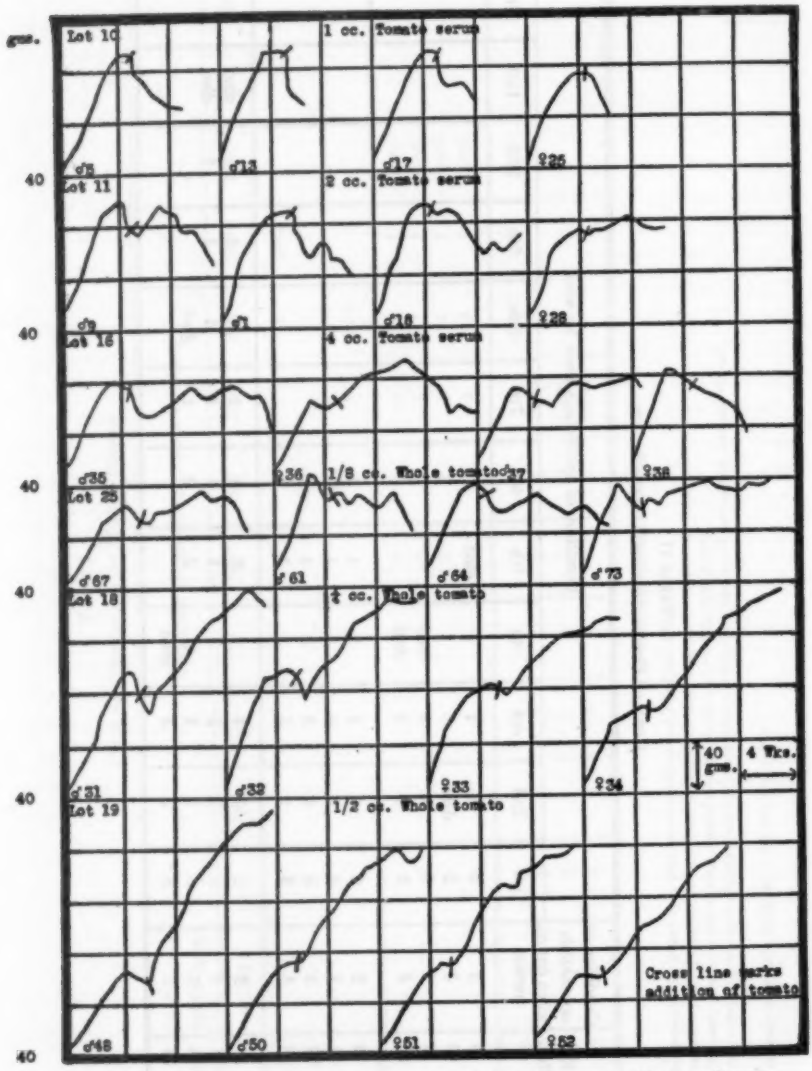

CHART. 1. GROWTH PROMOTING PROPERTIES OF WHOLE TOMATO VS TOMATO SERUM

Table I shows recovery from ophthalmia with the whole tomato preparation on 1/8 of a cc. daily at a very slow rate, but the amount of vitamin A contained in this daily supplement was not sufficient to maintain normal health. By the end of the ninth week all of the animals in this group had died. On 1/4 cc. tomato daily, the ophthalmia had been completely cured by the tenth week, and on 1/2 cc. the same result had been obtained by the eighth week and almost by the seventh. This proves beyond question the remarkable potency of tomato preparations in vitamin A, especially when it is remembered that this material contains only 4.44 per cent solids—which means that 1/4 cc. daily represented only approximately .16 per cent of the total daily intake of dry matter, or 11 mgs. daily.

Table II shows the results obtained with so-called tomato serum. It will be noted that on 1 cc. of this preparation daily, the rats had all died by the fifth week. On 2 cc. one animal died during the sixth week, and the others showed only a slight retrogression in the severity of their symptoms. On 4 cc. one animal died during the fourth week and the others improved very slowly, but ultimately died in the eleventh. Evidently other organs than the eyes were affected critically by the deficiency of vitamin A.

Comparing the results obtained on whole tomato with those on tomato serum, it is noted that the response with 4 cc. of the serum approximates fairly closely the response obtained on 1/8 cc. whole tomato. From this standpoint it may be concluded that the whole tomato contained about 32 times as much vitamin as the serum. Growth responses obtained by feeding the tomato preparations correlate very well with the ophthalmia indications. This is shown in Chart 1, which presents graphically the growth responses of the same animals of which the ophthalmic records are presented in Tables I and II. Maintenance on 1/8 cc. of whole tomato approximates very closely that observed on 4 cc. of tomato serum and 1/2 cc. of whole tomato led to approximately normal growth performance. Both growth and ophthalmic reactions show that in whole tomato there is approximately 32 times as much vitamin A as in the clear tomato serum.

The comparatively recent work of v. Euler and co-workers (38) found .005 mg. carotin per rat daily, effective as a source of vitamin A. Moore (26) confirmed the potency of carotin but Duliere, Morton, and Drummond (25) found no activity in a specially purified carotin. Lycopin, the red pigment of tomato, has been found inactive (39). We are quite certain as to the inactivity of lycopin because Mr. Baumann, in this laboratory, has found it inactive in dosages of .07 mg. daily in repeated experiments. With carotin purified by 20 precipations with methyl alcohol from chloro-

form solution in large volume, .007 mg. daily was found very active. However, the experience of various workers in connection with vitamin D naturally makes one rather skeptical as to whether the ultimate state of separation of a preparation into its physiologically active units has been attained not excluding the complicating possibility that a number of substances may elicit the same physiological reaction. We have found an abundance of yellow pigment extractable from tomato pulp with petroleum ether; the serum, however, contained but little.

Vitamin D

So far no mention has been made of vitamin D. As this is one of the vitamins upon which attention is widely focused, we obtained some data on its presence when we used tomato as a source of vitamin A in experiments which compared different cereals for their rickets-producing properties. We found that rats receiving additions of 2 cc. of tomato daily showed no increase in bone calcification. As it is difficult to give large quantities of tomato to rats, because they refuse to consume it, we later evaporated various amounts of tomato on our basal ration 2965 (40). This was done by the use of an air current at a temperature not exceeding 60°C. Ten, 20, and 40 cc. of tomato were evaporated on each 7 gms. of ration. These rations were prepared in quantity and then stored. They were fed in a series, together with ration 2965 as a control, to litter mates taken from four litters of rats weighing from 52 to 60 gms. This consumption was equalized so that by the end of the fifth week all the rats had consumed the same amount of ration. They were then killed and the fat-free femurs analyzed for ash.

The results are presented in Table III. They reveal an increase in weight

TABLE III

CALCIFYING ACTION OF TOMATO

Rat Nos.	Tomato Evaporated on 7 gms. ration	Food consumed	Tomato solids	Femurs weight	Femurs ash	Femurs ash
	cc.	gms.	gms.	mgs.	mgs.	per cent
65–68	0	1063	0	0.109	0.044	40.4
73–76	10	1048	66	0.127	0.055	42.8
77–80	20	1043	132	0.142	0.071	49.8
81–84	40	1064	269	0.164	0.089	53.6

of femurs, femur ash, and percentage of ash with increase in the amount of tomato in the ration. It is evident that with the consumption of large amounts of tomato, calcification of bone was improved and growth was

likewise improved. Without tomato the final weight of the animals was from 80 to 85 gms.; with tomato the animals weighed from 90 to 107 gms. It should, however, be mentioned that with such a large proportion of the solids consumed composed of tomato solids, which in one case amounted to 25 per cent of the ration, the calcium carbonate intake was reduced and the calcifying power of the ration was correspondingly improved. Taking this into consideration it is evident that the calcifying power of tomato actually is very low.

As tomato is rich in both vitamins A and C and therefore constitutes a valuable supplement for the diets of infants and children, it appears that it would be desirable in many instances to have it likewise potent in vitamin D. As the production and addition of vitamin D in the form of an irradiated ergosterol solution offers no special difficulties, we have carried out some experiments to determine if irradiated ergosterol would maintain its activity in such an acid medium as tomato represents. A determination of the pH of our tomato preparation showed it to have a value of 5.77.

Ergosterol prepared from yeast[2] was activated by exposure, in ether solution, to the radiations of a quartz mercury vapor lamp. After evaporation of the ether in vacuo it was brought into solution in alcohol and added to the tomato before it was bottled and sterilized in the usual commercial manner. One hundred fifty cc. of alcohol carrying 5 mgs. ergosterol were added to 15 quarts of tomato. This amount was much larger than necessary for the preparation of a commercial product of the desired potency, but it was added in excess in order that we could readily determine the percentage of destruction which might result on storage with the small amounts of tomato which rats might be induced to consume. We reserved part of the ergosterol for standardization, and other portions were added to tomato and then tested immediately before and after sterilization at 15 pounds pressure for one hour.

The bulk of the fortified tomato was placed in storage at room temperature in an incubator run at 37° and in a refrigerator. The potency of these was again determined after 2 and 13 months storage, using as controls some of the same tomato which had been freshly fortified with irradiated ergosterol, both sterilized and unsterilized.

The tests for potency were made by means of the Johns Hopkins line test technique (41) using rats of approximately 60 gms. in weight and 3 to 4 weeks of age. These were made rachitic by feeding for 3 to 4 weeks on

[2] The ergosterol was kindly furnished by The Fleischmann Co.

ration 2965. The tomato was then fed daily for 10 days in small dishes. At the end of the ten-day period the distal ends of the radii and ulnae were examined for calcium deposits.

The results after 13 months are presented in Table IV. Earlier results are not presented because they were identical with those given. All of them showed no loss of potency after storage even at 37° in an incubator.

TABLE IV

CALCIFICATION ON 0.1 CC. TOMATO PLUS ERGOSTEROL DAILY

Ergosterol	Litter No.	Rat No.	Initial weight gms.	Final wt. gms.	Average consumption R. 2965 gms.	Line Test
Freshly added	88	8938	88	105	11.0	+++
	89	8942	78	87	7.5	++
	90	9012	105	113	7.5	++
	91	9002[1]	96	106	6.7	++
	91	9006[2]	83	92	5.7	++
	96	9295	83	87	6.7	+
	96	9296	95	102	8.0	+
	4786	499	87	97	8.2	++
	4787	505	97	103	5.7	++
Freshly added and sterilized	90	9010	112	114	6.5	++
	91	9005[2]	88	93	6.6	++
Stored in refrigerator 13 months	4784	488	87	94	9.2	++
	4785	493	88	91	6.6	++
Stored at room temrature 13 months	4782	477	88	92	9.6	++
	4783	483	78	85	8.1	++
Stored in incubator 13 months	4780	466	89	79	6.4	++
	4781	671	89	100	7.0	++

SUMMARY

The literature on the vitamin content of tomato has been reviewed. Experiments designed to determine the distribution of vitamin A in red tomatoes showed that freed from skin and seeds the pulp contains approximately 32 times as much vitamin A as the clear yellow serum. From the nutritive standpoint, therefore, there is no justification for marketing a filtered tomato juice in preference to a juice containing the pulp in suspension. Vitamin D added in the form of irradiated ergosterol was found

to have maintained its activity after sterilization, followed by 13 months storage at 37° C.

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Editorial Review

THE FUEL OF MUSCULAR ACTIVITY OF MAN

THE subject of the source and kind of material which furnishes the energy for the performance of muscular work is one that has engaged the activities of many physiologists and students of metabolism. From time to time new studies appear which are either repetition of previous work by later and more accurate methods, or are actuated by new points of view which arise from new findings in physiology. The subject seemingly at times appears to be completely settled to the satisfaction of many physiologists, then a new investigation occurs with results which are not in line with either previous findings or with current theories. Thus a new impetus to investigation is given, and a renewed activity takes place in a field which has previously seemed to have been exhausted.

Chauveau (1896) made an observation upon Tissot of the respiratory quotient at intervals during 70 minutes going up and down stairs together with those of the rest periods preceding and following the period of activity. As the respiratory quotient rose from 0.74 to 0.75 at rest to 0.95 during work, Chauveau concluded that muscular activity was accomplished at the expense of carbohydrate, and that carbohydrate was formed from fat. Subsequently (1897, 1898) he made studies on a dog with diets of predominantly carbohydrate or fat and from studies of body weight changes, he concluded that fat was of nutritive value for muscular activity only to the extent that it could be converted into glycogen. Chauveau's researches have been criticized by Zuntz (1896, 1898) who, together with his associates, made a large number of studies upon the metabolism of muscular work with especial reference to the source of energy, and came to the conclusion (Zuntz, 1911) that during work the nutrients catabolized were essentially the same as during rest, and also that the oxygen absorbed per unit of work was essentially the same whether carbohydrate or fat was burned.

Benedict and Cathcart (1913) found that the respiratory quotient during work was influenced by the character of the preceding diet as the average quotient during work after a carbohydrate-rich diet was 0.90 and after a carbohydrate-poor diet was 0.82. They also found that, in general, the more intense the work, the higher was the respiratory quotient. They came to the conclusion that the values indicated that during the period of severe

muscular activity the character of the catabolism is changed by an increase in the carbohydrates burned, with a draft upon the carbohydrates of the body for such a purpose. At the completion of muscular work, the specific metabolism due to muscular work ceased, and the picture was one of a body subsisting upon a depleted storage of carbohydrate as shown by the lower respiratory quotient after muscular work.

Krogh and Lindhard (1920) made a very extensive study on the relative value of fat and carbohydrate as sources of muscular energy. The work was performed on a Krogh ergometer. The respiratory exchange was measured in an open circuit chamber apparatus, the outcoming air of which was analyzed by a gas analysis apparatus with an accuracy of 0.001 per cent. The subject usually rode the ergometer about two hours, and the measurements were made in three periods of 20 to 30 minutes after the subject had been riding for at least 20 minutes. The oxygen absorption varied between 860 and 1434 cc. per minute. In the three best series of experiments, the energy per unit of work varied from 4.6 calories at a respiratory quotient of 0.71 to about 4.1 calories at a respiratory quotient of 1.00. Their experiments led them to the following hypothesis: The proportion of carbohydrate to fat catabolized depends upon the relative available quantities of the two substances and is substantially the same during rest and during work. When the available supply of carbohydrate is in excess of that of fat, fat is formed from carbohydrate. This formation becomes distinct at quotients above 0.9. Sugar or allied substances are formed from fat (and protein) when the available supply of fat is in excess of carbohydrate. This formation becomes distinct below 0.8.

H. M. Smith (1922) determined by means of a closed circuit apparatus the respiratory exchange of men during grade walking. The duration of the periods was about 12 minutes and usually several periods were run in succession. The values of the respiratory quotient tended to increase as the grade and speed increased. The majority of the respiratory quotients up to approximately 600 kilogrammeters of work per minute were within the limits of 0.80 to 0.87 and for 700 to 1000 kilogrammeters of work per minute almost half of the respiratory quotients were 0.90 or above and none was below 0.85. For more than 1000 kilogrammeters per minute the respiratory quotients grouped around 0.93 and none was below 0.90. Two determinations with work greater than 1300 kilogrammeters per minute gave respiratory quotients of 0.97.

The author stated that short periods such as these with 600 kilogrammeters or less of work per minute did not tend to alter the character of the

respiratory quotient, but with moderately heavy or heavy work involving over 600 kilogrammeters per minute the body altered its metabolism by a tendency to a selective consumption of its carbohydrate reserve. These experiments definitely show a relationship between the character of the fuel for muscular activity and the intensity of the activity. In other words, the more intense the activity the higher was the respiratory quotient.

Furusawa, Hill, Long, and Lupton (1924) published a series of experiments on muscular exercise and the oxygen requirement. The study was principally to determine the oxygen requirement as distinguished from the oxygen absorption for different amounts of activity of very short duration consisting of walking, flat running, and standing running. In a group of 13 experiments in which the duration of the exercise varied from 16.3 to 18.3 seconds and the total duration of exercise and recovery varied from 6.2 to 10.6 minutes, there was a considerable variation in the total respiratory quotients and the respiratory quotients of the excess metabolism. The authors state that they were made before they realized the considerable duration of the recovery process after severe exercise. In several of them they say that the recovery was not quite complete and the oxygen requirement as determined was appreciably too small.

In two succeeding tables they give 11 experiments on K. F. with the particular object of determining the oxygen requirement. In the discussion of the results of these experiments they point out that the excess quotient, *i.e.*, the quotient of the excess carbon dioxide and the excess oxygen over the base line due to work and recovery, varied from 0.93 to 1.13 with an average of 1.02 in one group and 1.04 in the second. They state that the interval allowed for recovery was admittedly not long enough for complete recovery.

The authors concluded that with man undergoing prolonged exertion the respiratory quotient certainly is not unity. "It would seem possible, however, that in man an element of muscular exercise, of such short duration that it does not seriously affect the general metabolism of the body, may be paid for primarily by the oxidation of carbohydrate and that if the effort be prolonged the carbohydrate oxidized must be restored at the expense of other substances." They were inclined rather to regard the high respiratory quotient of the recovery process of these short-lived efforts as a genuine effect. They stated, however, that the conclusion was so important that more careful work was needed with longer recovery periods and that at that time the conclusion must be regarded as tentative. These experiments were made later by Furusawa.

Furusawa (1925) made the most striking observations upon the character of the metabolism due to short periods of muscular work. The exercise of standing-running was employed. The subject rested for 20 to 30 minutes, during which period the author states that the recovery process from previous muscular activity should be completed. After a 10-minute sample of expired air was collected which gave the first base line value, the exercise was taken and the expired air from the beginning of exercise to recovery was collected in a large Douglas bag. Another 10-minute collection of expired air was taken after recovery which gave the second base line value. These two values gave the basis from which the excess metabolism was calculated.

The first group of Furusawa's investigation contained 13 experiments on two subjects with periods of exercise from 0.5 to 1.0 minutes, with a total time of collection of 10 to 40 minutes. The basal respiratory quotients varied from 0.81 to 0.93. The respiratory quotient of the excess metabolism was close, with few exceptions, to unity. The extra work involved, however, was small so that the maximum amount of carbohydrate catabolized during the period of 40 minutes, including that due to basal metabolism, was 10.7 grams and of this 6.3 grams was due to the excess metabolism. The minimum total carbohydrate catabolized in the work and recovery periods was 1.7 grams and the minimum excess metabolism of carbohydrate due to exercise was 0.4 gram. The oxygen requirement for the work varied from 700 to 9000 cc. per minute. The second group consisted of 4 experiments with long-continued exercise from 10 to 30 minutes and the time of collection varied from 55 to 70 minutes. The total carbohydrate used in these cases varied from 17 to 35 grams and the carbohydrate in the excess metabolism due to exercise from 9.8 to 23.4 grams. The greatest deviation from unity in the respiratory quotient of the excess was 0.04. The average oxygen intake was of the order of 700 cc. per minute. The author stated that in the 30-minute exercise 140 grams of carbohydrate must have been oxidized. (This is evidently an error; presumably 140 calories was intended.)

The next group contained several experiments at higher speed with an oxygen requirement of 1.9 liters per minute. Here again the greatest deviation from unity was 0.06 for the excess metabolism and the total carbohydrate metabolism varied from 39 to 56 grams in exercise and recovery, whereas the excess metabolism of carbohydrate varied from 29.9 to 49.5. The respiratory quotient of the excess metabolism in the 28- and 30-minute experiments was 0.94 and 0.88. According to the author, only following the

oxidation of 300 grams of carbohydrate could the oxidation of fat be detected. (This is obviously an error.)

A group of 12 experiments was performed in which the diet preceding the experiments was mainly fat. The basal quotient varied from 0.65 to 0.77. The time of exercise was from 0.33 for 272 steps per minute to 2.0 minutes at 146 steps per minute. The time of collection varied from 20 to 35 minutes. The carbohydrate in the total metabolism of exercise and recovery in this group was from 1.4 to 8.2 grams, whereas the carbohydrate of the excess metabolism due to exercise was from 1.1 to 6.3. In spite of the low initial quotient the respiratory quotient of the excess varied from 0.98 to 1.06. The single experiment in which the base line quotient of 0.65 was found is given in somewhat more detail and it is shown that the respiratory quotient both before and after the exercise as well as the carbon dioxide and oxygen were nearly identical.

Furusawa concluded that the metabolism due to a short element of exercise was the same, namely, the oxidation of carbohydrate only, whether a great amount of carbohydrate or only fat is supplied to the body. This suggested that the position of carbohydrate in muscular work was unique, and that fat was used only after conversion into carbohydrate.

A fifth group of experiments after a high fat diet at 146 steps per minute with a basal quotient from 0.72 to 0.76 and a duration of exercise from 4 to 9 minutes gave as a respiratory quotient of the excess from 0.92 to 0.97. In this group the carbohydrate metabolized in the total period of exercise and recovery varied from 12.9 to 23.0 grams and the excess metabolism varied from 11.5 to 19.5 grams.

The author concluded that with short-lived muscular exercise even when the basal respiratory quotient reached 0.71, the exercise was performed at the expense only of carbohydrate. From these facts it could be concluded that with exercise of short duration in which no change in the general metabolism of the body as a whole might be expected, the human body acted as though it were an isolated muscle in which carbohydrate only was oxidized. The primary fuel of contraction, therefore, in the human muscle is carbohydrate, and fat or protein is presumably used to replenish the carbohydrate store which has disappeared.

From a general survey of Furusawa's experiments it would appear to the reviewer that when the diet was normal, there was enough available reserve of carbohydrate to draw upon for these short periods of exercise, so that there was no need to call upon fat even in the long-continued experiments of 30 minutes' duration where a total of 35 grams of carbohydrate

were used in a period of 70 minutes. It was only when the draft upon carbohydrate was about 40 grams, that the call upon fat was shown. In contrast to the experiments with normal diet are the experiments with fat diet in which small quantities of carbohydrate for the most part were catabolized during the period of work and recovery or due to the work itself, but here the draft upon fat was at a lower level of carbohydrate catabolized than after normal diet as it began at apparently 12 to 13 grams of total carbohydrate catabolized. In the opinion of the reviewer, the results in the experiments of Furusawa were due to the small amount of work involved and to the fact that even on a fat diet there was still enough carbohydrate available to supply the energy for exercise and recovery.

Hetzel and Long (1925) determined the respiratory exchange of three diabetic patients before, during, and after muscular exercise. They used the same methods that were used by Hill and co-workers. The observations were divided into three groups depending upon the time of the last injection of insulin: 1—insulin injection within the last 6 hours; 2—without insulin for periods from 10 to 17 hours; 3—without insulin from 20 to 27 hours. The exercise was that of "standing-running." The preliminary period varied from 20 to 60 minutes during which the subject rested, then a collection for the resting sample of from 10 to 15 minutes was made. At the end of the recovery another resting sample was collected from 10 to 15 minutes and, if the initial and final resting metabolisms did not agree within reasonable limits, the experiment was discarded. The time of recovery was from 30 minutes for the half-minute periods of exercise to two hours for the 8-minute periods.

In the exercise of short duration with insulin taken within the last 6 hours or within the last 10 to 17 hours, the average of the respiratory quotient of the excess metabolism in 17 experiments was 0.99 as compared with a previous average respiratory quotient of 0.76. In a second group in which the exercise was of longer duration with a recent administration of insulin, the average respiratory quotient of the excess metabolism was 0.85 as compared with a resting quotient of 0.76. The authors calculated that 15 per cent of the energy at rest was derived from carbohydrate, whereas during work 50 per cent was derived from carbohydrate.

In the third group with exercise of short, moderate, or long duration without recent administration of insulin, the average respiratory quotient of the excess metabolism was 0.80 as compared with a previous resting value of 0.76.

The authors concluded that in the absence of insulin the respiratory

quotient of the excess metabolism apparently is never unity, however short the interval of exercise, and that the results of diabetic patients without insulin represent an exaggeration and extension of the results found with normal men on a fatty diet. They believe that the carbohydrate is restored by the transformation of some other substance before the recovery is complete, that instead of the muscles being able to use fat directly, it is simpler to suppose that they can use carbohydrate only and that insulin is necessary only in order to maintain the carbohydrate at sufficiently high levels for use in oxidations in recovery. Rapport and Ralli (1928) stated that they were unable to agree to the above interpretation of these results, because, if true, one would expect increasingly lower quotients of the excess metabolism as the exercise was prolonged, but their analysis of Hetzel and Long's results did not show such decreases in the quotient.

Henderson and Haggard (1925) studied the respiratory exchange of a group of Yale rowers during and subsequent to a period of exercise on a rowing machine. The expired air was collected in a spirometer of 400 liters capacity and two large Douglas bags and the analyses were made by a Henderson-Orsat apparatus. The subject removed his outer clothing and, after adjusting the foot board, sat quietly on the sliding seat of the rowing machine for 10 minutes. After this partial rest, his expired air was collected for five minutes. He then rowed at a uniform rate for 5 minutes. The whole expired air for this period was collected. Then he sat still for fifteen minutes during which the expired air was collected in a Douglas bag for two minutes, then three minutes and then ten minutes.

The authors concluded that the values found showed that whatever proportion of fat and sugar the subject burned during rest prior to work, he continued to consume during a short period of great exertion. Sugar was therefore not the sole fuel of muscular energy.

The respiratory quotients at rest before work were extremely variable in this group of subjects, from two at 0.66 to 0.67, which the authors questioned, to one at 0.94, including one each at 0.75 and 0.74. In the great majority of cases the respiratory quotient during five minutes of work was higher than the quotient of the rest periods. Similarly, this was true with regard to the two minutes of recovery and the next three minutes of recovery. The respiratory quotient of recovery and work was higher than the rest quotient in 8 out of 12 experiments and the respiratory quotient of the excess varied from 0.72 to 0.96, and was higher than the rest quotient in 8 out of 12 cases. The total carbohydrate used in these experiments in work and recovery varied from 2.2 grams with one subject, whose basal

quotient was 0.73, to 24.1 grams. These are not, therefore, extraordinarily high values. If one of the conditions for securing a respiratory quotient of unity for the excess metabolism due to work is that the draft upon carbohydrates shall be small, we would expect a respiratory quotient of unity in this group. However, in the opinion of the reviewer there were several factors in this investigation which would hardly conform to the conditions which are optimal for obtaining a respiratory quotient of unity due to work. The conditions for obtaining the pre-work quotients were not ideal. After a ten-minute rest one could hardly expect to obtain the real base line quotient particularly after the muscular activity involved in undressing and adjusting the apparatus. In addition, the recovery periods were not continued long enough to approach the values of the rest period. The last oxygen value was frequently over 100 cc. higher than the pre-work value, and, therefore, the subjects had not recovered and the experiments did not conform to the requirement. The minimum difference between the pre-work value and the last recovery value is 50 cc. The experiments of Henderson and Haggard indicated that in the majority there was an increase in the proportion of carbohydrate utilized in the period of exercise and recovery.

Wilson, Levine, Rivkin, and Berliner (1927) studied the effect of moderate exercise upon the respiratory quotient with four children in which a closed circuit chamber respiration apparatus was used. In one group, the rest and exercise periods were each of two hours' duration and were made on the same day on two of the subjects and on different days with the other two. In the experiments with exercise the precaution was taken to have the exercise stopped before the end of the period in order to allow conditions in the chamber to reach a steady state. This period was from 30 to 25 minutes before the end. The respiratory quotients during the rest experiments and the experiments with exercise were nearly identical. The percentage increase due to the activity varied from 17 to 52. The respiratory quotient of the extra metabolism varied from 0.71 to 0.87 and in nearly all cases was very close to the respiratory quotient of the rest experiments. The authors concluded that the results appeared to furnish further evidence in favor of Lusk (1925) and his co-workers that fat can be burned directly under stress.

The reviewer is of the opinion that a closed circuit chamber respiration apparatus is not suitable for measuring the change in the respiratory quotient due to such small increases in metabolism, especially when the rest and exercise experiments take place on different days.

Marsh (1928) made an extensive study of the fuel of muscular activity during work and recovery. Five boys were used as well as four adult laboratory workers and seven medical students. With one of two boy subjects used extensively no attempt was made to control the diet or activity. The other boy ate a standard breakfast and lunch on the experimental day. The respiratory exchange was measured by means of the Benedict Universal apparatus. The work was performed on bicycle ergometers. The author states it is quite as necessary to secure recovery of the original rate of carbon dioxide elimination as to secure recovery of the original rate of oxygen absorption and that results would not be accurate if the recovery was not complete, or a diminished respiratory exchange followed immediately upon complete recovery.

The subject rested 20 minutes to one half hour. He then took his position upon the bicycle, adjusted the mouthpiece and noseclip and, after the breathing had become regular and normal, the first resting period of 7 to 10 minutes was made in this position. After the rest period, the subject was disconnected and sat in a chair while the absorbers were being weighed and then followed the work and recovery periods with no interruption between them.

The respiratory quotient of the excess was obtained by deducting from the carbon dioxide and oxygen of the work and recovery period the values of the first rest period. It was found that no material difference was made in the average values whether the first rest period or both rest periods were used in the calculation. The author points out that there may be overventilation at the beginning of work but, if the recovery period is sufficiently long, a period of retention of carbon dioxide would in time balance the blowing off and thus restore the equilibrium. It is essential that the recovery be continued to this point as well as to the point where the rate of oxygen absorption equals that preceding the work period.

The author made a special series of experiments to determine the length of time for recovery and she found that it was possible to continue too long, because it led to an irregularity which might be explained by the unrest caused by sitting on the ergometer. She came to the conclusion that a period of only 8 minutes was sufficient for the work which was done by this subject, a boy. A series of 37 experiments was made with one boy in which the average basal quotient was 0.87 and the average quotient of the excess metabolism was 0.78. In another group of 11 experiments with the same subject, the average basal quotient was 0.91 and the excess quotient was 0.88 and in general on a much higher level than the earlier series with

the same subject. The results in the latter group are ascribed to the subject's better physical condition.

With one adult subject three types of experiments were made, one with a normal diet, another with a high carbohydrate diet, and a third with a high fat diet. The first group consisted of 16 experiments on a normal diet. Half the experiments were of 5 minutes' duration of work with 8 to 12 minutes for recovery and the other half 5 to 10 minutes work with 11 to 17 minutes for recovery. The work ranged from 4.48 to 13.7 calories. The average respiratory quotient of the excess metabolism was 0.95, varying from 0.88 to 1.05, and the average basal quotient was 0.82 with a range from 0.75 to 0.86. Part of the experiments were in the post-absorptive condition and part from 3 to 5 hours after breakfast. This latter condition did not seem to make any positive difference in the pre-work respiratory quotient or in the respiratory quotient of the excess metabolism. The author states that the high respiratory quotient in the work and recovery may be associated with the subject's physical condition and perhaps state of maturity as compared with the boys'.

In the first group with the carbohydrate diet the average respiratory quotient before work was 0.83 and the average respiratory quotient of the excess was 0.96 with a range from 0.88 to 1.05.

In the first group on a fat diet, the base line quotients averaged 0.73 with a range from 0.70 to 0.80, thus showing definitely the effect of the diet upon the quotient. The excess quotient, however, was 0.83 with a range from 0.79 to 0.86. Thus in spite of the fat diet, there was some carbohydrate burned during muscular work. The recovery was apparently slower on the fat diet both as regards carbon dioxide and oxygen even when the period was continued as long as 28 minutes.

A carbohydrate series followed with results essentially similar to the first series. The average respiratory quotient of the excess metabolism in 10 experiments was 1.00 and the quotient before work was 0.88 and 0.91 in the two groups of this series. This was succeeded by another series on the high fat diet. The average basal quotient was 0.74 ranging from 0.71 to 0.80. The quotient of the excess metabolism was 0.80 and the average quotient after the work ceased was 0.81. This seems singular because as the muscular work caused a greater proportion of carbohydrates to be metabolized than during rest, one would expect that the quotient after the work ceased would be as low if not lower than the quotient preceding the work. This may have been due, as the author points out, to the fact that the recovery of the carbon dioxide to resting value was not obtained.

The author concludes that the response to the work varied with the individual and that there does not seem to be a uniform response to muscular work in so far as the change in respiratory quotient is concerned.

This study is of interest to the reviewer primarily in connection with the study on the adult in which a long series of experiments was made. The conditions for obtaining changes in the respiratory quotient due to the effect of small amounts of work were not ideal, in the first place because the base line period was obtained immediately after the subject had changed his position, in the second place because the recovery period was obtained in a condition which ultimately led to discomfort and in some cases to a shifting set of recovery values, as pointed out by the author.

The group of experiments on the adult is consistent in that both carbohydrate groups gave high respiratory quotients in the excess metabolism as well as with the normal diet and the experiments with the fat diet pointed in the same direction although not so positively as the carbohydrate diet. In other words there was a definite increase in the carbohydrate metabolized due to muscular work even after a fat diet which resulted in a very low base line quotient.

Lindhard (1928) sought to test the general thesis of A. V. Hill and coworkers that a short period of muscular work proceeded exclusively at the cost of carbohydrates. He criticized the series of experiments on the material upon which Hill and his co-workers (Furusawa, Hill, Long, and Lupton, 1924) drew their conclusions. He particularly called attention to the variations in respiratory quotient of the work ranging from 0.93 to 1.13 with an average of 1.03. He referred to other tables in the same article that had a wide variation in the respiratory quotient of the excess metabolism due to work and that the authors do not mention these experiments. He illustrates the fact that with a very moderate amount of work the respiratory quotient of the excess metabolism is not unity but below unity. However, in this connection it must be mentioned that the authors themselves stated that those experiments were carried out before the necessity for a longer recovery period was recognized and even in the experiments from which the average quotient of 1.03 is drawn, they stated that the period of recovery was too short, that the quotients were likely to be too high, but that the evidence pointed in the direction of carbohydrate being a source of muscular work when very short periods of work are performed and that the idea was wholly tentative with experiments planned in the future. Lindhard also criticized as worthless the work of Simonson (1926) in which a range of quotients for excess work from 0.71 to 1.31 was found

with an average of 0.99. Simonson's studies were made with the Zuntz-Geppert apparatus. It is a pity that this serious and conscientious worker should have published so much material based upon a technique wholly inacceptable to research workers in this field.

Lindhard's experiments were performed with a Krogh ergometer with 60 revolutions per minute and a range from 892 to 1115 kgm. per minute. The expired air was collected in Douglas bags suspended on a wire net above the subject. The subject came to the laboratory in a post-absorptive condition and sat immediately and began shortly to breathe through a mouthpiece and valves, filling the bags which were to be used for the experiment. After one-half to three-quarters of an hour the subject sat on the ergometer and after another 15 to 20 minutes, he applied the nose clip and mouthpiece and breathed to the atmosphere for 20 minutes before beginning the rest experiment which was six to seven minutes' duration. Several minutes after the rest experiment, the work experiment began. Three bags were used for continuous collection of expired air during the work period and recovery. If more than three were necessary there was a pause between the last and the fourth.

Sample experiments are shown graphically in which the course of the oxygen absorption and carbon dioxide are given by periods during work and recovery. He then discusses the advantages and disadvantages of the method and the fact that because of the limit as to the length of time during which the subject could remain upon the ergometer during the recovery period, breathe through the mouthpiece and still remain comfortable, the recovery period was of necessity shortened.

Lindhard's method of application of the periods of recovery was different from that of Hill because he included only the periods of recovery to the point where the respiratory quotient fell to the respiratory quotient of the pre-work period without reference to the fact that the respiratory quotient may rise to a higher level after this point is reached. In other words, during the recovery he includes only those periods up to the point where the line showing the course of the respiratory quotient first cuts the value of the base line.

He had 12 experiments with two subjects in which the basal respiratory quotients before work varied from 0.71 to 0.88 and he states that particularly with one subject in which the range was the same, these variations were real, due to the training and to the variation in diet. A quotient of 0.71 seems to the reviewer unusually low for a subject on an ordinary mixed diet and we would look with doubt upon the quotient below 0.76 un-

less the subject had been without food for more than 12 hours and had had a low carbohydrate diet. The range of the respiratory quotients in the rest period after the work varied from 0.74 to 0.89 and in most cases was slightly higher than the respiratory quotient before the work. His comment regarding this is that it was only apparently the case because these numbers refer to the middle of the period and if one takes the end of the line where the curve of the quotient cuts the rest level one will see that the real quotient at the end of the rest period corresponds to that before the work.

The total excess metabolism varied from an oxygen consumption of 1938 cc. in 12.79 minutes to 64.7 liters in 50.5 minutes. There was thus an extremely wide variation in the extra metabolism. Similarly the work period varied from 1.93 to 36.28 minutes. The recovery periods were short, from 10.62 to 24.32 minutes. The respiratory quotient of the excess metabolism varied from 0.82 to 1.205 and the majority of the quotients are above 0.90. There was a wide range in the total carbohydrates catabolized during the work period and recovery period. The variation was from 1.6 in 14.6 minutes to 59.2 grams in 51.2 minutes. Lindhard stated that these were typical experiments.

There is a uniformity neither in the basal respiratory quotients nor in the respiratory quotients at work, nor is there any parallelism in the gradual changes. In three of the experiments, the second rest value had an oxygen absorption from 35 to 65 cc. per minute higher than the first resting periods. With conditions so variable and with results also so variable, in the opinion of the reviewer it is questionable whether these experiments are of significance in solving the question of the source of energy for muscular activity of short duration.

Bock, Vancaulaert, Dill, Fölling, and Hurxthal (1928) made a study of the respiratory quotient during work, particularly with reference to the steady state. According to them, the steady state may be associated with an oxygen debt provided it is not cumulative, but is principally acquired in the period at the beginning of work. A steady state cannot be maintained by the average man not in training for a continuous period of work on a stationary bicycle when the oxygen requirement of the work done exceeds about 2 liters per minute. Some of their recent experiments running on a treadmill indicate that an oxygen intake of 2.5 liters per minute can be continued for 2 hours with this type of exercise.

Two experiments were made with the subject D. B. D. The metabolic rate was nine times that of the resting state. The lactic acid and total

carbonic acid of blood returned to a resting level at the end of 60 minutes of work. The average respiratory quotient for the total metabolism was 0.96 and for the excess metabolism was 0.97. Another experiment on the same subject in the post-absorptive condition showed that a steady state was reached at the end of 4 minutes and at the end of work nearly basal conditions were obtained in fifteen minutes. Most of the excess oxygen taken in during this period was due to the oxygen debt acquired in the first three minutes of work. A small part of it might be referred to the gradual return of the machine to the state existing before the work was begun. The average respiratory quotient of the total metabolism was 0.93 and of the excess metabolism in the working period was 0.97.

Tables are given showing the respiratory quotients for the total metabolism from the beginning of work until the end. With D. B. D. the R. Q. began at 0.98 and fell to 0.88 in the 50 to 59th minute. With A. V. B. the fall in 44 minutes was from 1.02 to 0.88.

The authors adhere to the following hypothesis with regard to the significance of the respiratory quotient as a means of interpreting the processes of catabolism. The primary source of energy for muscular contraction in earlier stages of work is carbohydrate. During moderate work glycogen is utilized, but at the same time the velocity of reactions involved in its synthesis from fat or other processes by which other sources of energy may be utilized nearly keep pace with the rate of glycogen utilized. The resultant respiratory quotient will rise only slightly above the resting level. With more severe work the muscles utilize stored glycogen with a velocity of the first order and the resultant respiratory quotient approaches unity. Time relations are now such that the conversion of fat to carbohydrate of a velocity, say of the second order, has no effect upon the respiratory quotient. The quotient will remain high until the glycogen is considerably lowered. The depletion of readily available glycogen results in speeding up the reaction fat to glycogen, or whatever other processes lead to the utilization of other substances, and the quotient reflects this reaction. The authors stated that the strongest argument in favor of the view that glycogen is the invariable source of muscular energy seemed to them to depend upon the consideration that the intrinsic physico-chemical processes involved in muscular contraction are always the same. Hence, it seems probable that whatever different sources may involve the process of muscular contraction, the chemical process through which all substances pass must be in part common to all of them. They believe that glycogen or some substance

very closely related to it in normal metabolism is the immediate source of the energy of muscular contraction.

The most extensive series of experiments on humans in the study of the respiratory quotient of excess metabolism of exercise was made by Best, Furusawa, and Ridout (1929) over a period of two years. One group of experiments was made in Toronto, another in Ithaca, and the third in London. The authors prescribed the following precautions for obtaining reliable and significant results. The period of rest should not be less than thirty minutes during which the mouthpiece or similar apparatus should be worn, *i.e.*, breathed through. They advise collecting the whole of the expired air in one bag or suitable container, or if collection is made in several portions, preventing escape of any of the expired air. The period necessary for recovery cannot be guessed but must be determined by experiment. Unless recovery is known to be complete, the respiratory quotients have no meaning except in so far as they show the production and removal of acid in the body. A practiced and suitable subject should be used. The authors state that to employ a subject showing irregular results is not to prove that any foodstuffs may be used, but rather a sign of insufficient experimental precautions.

The first group of 23 experiments was made at Ithaca, New York, on sprint running at absolutely top speed. The excess respiratory quotients varied from 1.18 to 1.68 and in the majority, the respiratory quotient of the total metabolism of activity and recovery was nearly unity. They consider two possibilities for the causes of the results, first, that excess carbon dioxide was blown off with consequently too short a recovery process, second, that the resting metabolism had been changed as the result of violent exercise, and that more carbohydrate was used during the basal oxidations proceeding at the same time with recovery process than during the same oxidation before exercise. If the first suggestion were correct, they would expect a retention of carbon dioxide in a later period of recovery which should lead to a lower respiratory quotient in the second resting determination. There is some evidence of a lower final quotient in 11 experiments, but the quantity of carbon dioxide still being retained was far too small to compensate materially for the large quantity which had been expired in excess. Moreover, in the other experiments the respiratory quotient in the second rest period was equal to or higher than that of the first. They were dealing with such large excesses of carbon dioxide that the small fall of 6 cc. per minute would require several hours for the retention of the extra carbon dioxide which had to be accounted for. In order to test the question

as to whether it was a washing out of carbon dioxide, they had several sprint runs with recovery periods in between each pair of runs. The results were the same as when only one run was made. Excess carbon dioxide was found after the second and after the third run, and it seemed therefore inconceivable that the extra carbon dioxide was due to excessive respiration and acid formation. The total extra carbon dioxide to be accounted for would make an excess of about 10 liters, the equivalent as an acid of 40 grams of lactic acid. This amount of lactic acid over and above that found normally at rest would cause a rise in the blood and soft tissues to more than 110 milligrams per cent, a value which is about the maximum found after exercise.

Another group was obtained by Furusawa in London with standing running at an extreme speed of 260 steps per minute. Forty to 60 minutes were allowed for the collection of expired air after exercise. The respiratory quotient of the total metabolism was about unity and the excess metabolism had a respiratory quotient of 1.19 to 1.68. Further experiments at Toronto by Best and Ridout confirmed these results. The time allowed for recovery was 50 minutes.

In order to determine whether there was a washing out of carbon dioxide at any stage with a subsequent retention, the recovery period was divided into several short periods. There was, however, little evidence of a retention of carbon dioxide or fall of respiratory quotient. In the blood and tissues lactic acid may displace an equivalent amount of carbon dioxide. The calculated quantities of extra carbon dioxide were from 1.8 to 2.5 liters with a corresponding expected presence of 7.3 to 10.6 grams of lactic acid supposedly at the end of an hour's recovery from exercise lasting only half a minute. These are impossible amounts. In addition, the same process could be repeated again and again. A direct determination of the lactic acid content after exercise and recovery proved that there was no important increase in the lactic acid content of the blood.

A group of 7 experiments with more moderate activity was conducted at Ithaca, and showed that the respiratory quotient of the excess was very close to unity. Similar observations made in London confirmed this series and a group at Toronto on standing running at moderate speed, and with riding a bicycle ergometer at slow speed, showed essentially the same results.

A group of 29 experiments with standing running at low speed stepping low in Toronto showed consistently that the excess respiratory quotient

was lower than unity. In fact, some of the values were nearly equal to the basal respiratory quotient.

The authors stated that it might be assumed that the excess metabolism is only such a small fraction of the total metabolism in the case of mild exercise that small errors in the estimation of the quantities involved might lead to erroneous results. The large number of observations, however, and their consistency seemed to weaken this objection. There was a difference between the two subjects as to the level of exercise at which the respiratory quotient rose to unity. In the case of C. H. B., an oxygen requirement of 1.0 to 1.9 liters per minute showed the lower level of the excess respiratory quotient, whereas, E. Mc H. exhibited a value nearly unity at an oxygen requirement of 0.7 liter per minute.

A group of experiments was conducted by Furusawa in London with moderate exercise with values varying from 0.74 to 1.04 liters of oxygen for the excess metabolism. According to the authors, where the oxygen requirement of exercise was less than 0.5 liter per minute, a small error in the resting metabolism of 2 or 3 cc. per minute could have made a large error in the respiratory quotient of the excess metabolism. Experiments were performed with longer resting collection of from 40 to 60 minutes, but this made no difference in the results.

The authors conclude that the high excess respiratory quotient was really caused by some metabolic change induced by very violent exercise. The results showed clearly that in the case of mild or very mild exercise, carbohydrate is not the sole foodstuff involved in muscular exercise. The respiratory quotient of the excess metabolism accompanying exercise did not invariably attain the value of unity. It is a function of the oxygen requirement rising gradually from the level of the basal metabolism to a very high value. It seems impossible to explain this gradually changing nature of the excess metabolism, especially the high values observed, by any such simple assumption as the combustion of carbohydrate and the washing out of carbon dioxide, or by the hypothesis that the body can utilize any foodstuffs available in order to provide energy for muscular contraction. They then discuss the possibility of an excess mobilization of sugar with a subsequent conversion to fat. However, they did not obtain any evidence of a liberation of sugar in their experiments and they did not, therefore, feel justified in this explanation.

They state that the small increase observed in the total respiratory quotient in very moderate exercise indicated that the body was able to speed up the preparation of carbohydrate from other materials to meet the in-

creased demand. In this case, the new formation could keep pace with the rate at which the muscles consume carbohydrate and the effect on the respiratory quotient is that any foodstuffs could be used directly to provide energy for muscular contraction. With increased severity of exercise the demand for carbohydrate increased and the process of new formation was no longer adequate to meet the demand. The increased combustion of carbohydrate may then overshadow the effect due to the contemporary manufacturing process. Consequently the stage at which the respiratory quotient of the excess metabolism reached unity was arrived at, and finally extreme violent exercise induced a totally new phenomenon for which no satisfactory explanation exists.

Their investigation is the most complete series of measurements on the metabolism of exercise and recovery thus far available. It has the great advantage that the several series were conducted in three different places by different observers and therefore there is no question involved of the use of one instrument throughout or only one observer. The group of experiments in which the respiratory quotient of the excess metabolism was over unity form the marked exception to all other investigations. It would seem to be most important for this type of study to be repeated by other observers. At present there seems to be no satisfactory explanation for this phenomenon.

Carpenter and Fox (1929) determined the respiratory exchange of a subject who rode on a Krogh ergometer for one hour at 275 kg-m. per minute and for one-half hour at 555 kg-m. per minute. The work period was preceded by a base line measurement of four 15-minute periods in succession and the periods of work were followed by seven 15-minute periods of recovery after the one hour's work and nine 15-minute periods of recovery after the one-half hour's work. The measurement of the expenditure due to work included the act of rising from a chair, mounting the ergometer, and the recovery periods included dismounting the ergometer and returning to the chair. The measurement of respiratory exchange during the work and recovery periods was continuous and was made with an open circuit apparatus with which a mouthpiece and valves were used. The respiratory quotients of the base line for the two groups varied from 0.78 to 0.87. The oxygen absorption during the periods was approximately 1000 cc. per minute in the one hour's work and in the one-half hour's work was approximately 1500 cc. In the one hour's work, the average respiratory quotient of the excess metabolism was 0.89 with a variation from 0.93 to 0.86 and for the one-half hour's work, 0.95. Thus in both groups the metabolism of

excess was higher than the base line and in the case of the more intense work of one-half hour was not far from unity. Two groups of experiments with the same amount of work and conditions of measurement were made in which 50 grams of glucose were given just before beginning the work. The respiratory quotients in the base line periods averaged 0.83 for the one hour's work and 0.82 for the one-half hour's work. The respiratory quotients for the excess metabolism averaged 0.96 and 0.99. When the excess gaseous metabolism was corrected for the increases in carbon dioxide and oxygen absorption due to the ingestion of 50 grams of glucose at rest, the respiratory quotient of the corrected excess metabolism was 0.94 and 0.98, thus higher than the excess quotients obtained when no sugars were given under the same conditions of work and recovery. Therefore, when glucose was given simultaneous with work, at least part of the ingested glucose was utilized in the performance of work.

These experiments differed from most work of similar nature by others in the long basal period. Care was also taken that the subject would be in a position comfortable enough so that he could relax completely after the work was done.

The results obtained by various investigators vary considerably. Some of these variations must be ascribed to the conditions under which the experiments were performed. One of the most important measurements is that of the basal metabolism or base-line both from the standpoint of total oxygen absorption and from the standpoint of the respiratory quotient. The conditions of the subject with respect to posture, bodily comfort, and possibility of complete relaxation under which he is measured should be the same as those that he is likely to reach after the work and recovery periods are finished. It must be obvious that a measurement of the R.Q. made after a shift in position without a sufficient length of time for adjustment cannot be considered reliable. The practice of having the subject breathe through the breathing appliance during at least one quarter hour and preferably the whole of the rest period before a measurement is made is a good one. This gives opportunity for adjustment of the organism to the new conditions of respiration and the subject is more likely to give a true respiratory quotient as well as normal oxygen absorption after a breathing period than after insertion of the mouthpiece within two or three minutes of taking a position. It would be advisable to have more than one period, if possible, in succession without an interval, because then there is no opportunity for compensation. There does not seem to be any reason for having the exact amount of work performed as one can obtain an estimate upon

this from the oxygen requirement. The condition to which the subject will return after the work is finished should be one in which it is possible to measure the respiratory exchange indefinitely or at least for two or three hours. The measurement of a recovery period while the subject is sitting upon a bicycle ergometer does not seem to be a practical one so far as complete relaxation is concerned, and it would seem as though it was necessary to continue the recovery period until the oxygen absorption had returned to the pre-work value or to a base line which is constant for several periods in succession. If the work has been so intense and stimulating as to cause a rise in metabolism which persists for some time, it is obvious that one cannot expect a return to the pre-work level in the oxygen absorption. Some investigators have laid down the condition that the recovery period should end when the pre-work respiratory quotient has been attained. This, however, must of necessity come within a very short period after the work finishes as the fall to a low quotient is rapid in the recovery period after work is finished. Subsequently there may be a rise which probably more truly represents the actual respiratory quotient after recovery is complete than the point at which the respiratory quotient reaches the pre-work level for the first time. If the work has been continued long enough and is exhausting enough, it is obvious that the respiratory quotient may not return to the pre-work level again until food is furnished, and therefore there cannot be a return of the carbon dioxide as well as oxygen to the pre-work level.

The technique of the determination and the physiology of the respiratory quotient has recently been treated very thoroughly by Richardson (1929).

The best apparatus for measuring the respiratory exchange and determination of the respiratory quotient is one of an open circuit type in which the outgoing air is analyzed by some form of Haldane apparatus, if the breathing applicance is a mouthpiece, and by the gas analysis apparatus of Carpenter (1931), if it is a helmet device such as recently described by Benedict (1930). The helmet undoubtedly furnishes the optimal conditions for normal breathing and it is possible to continue measurements of the respiratory exchange over long periods of time with little or no discomfort to the subject. The closed circuit principle for such types of work does not seem the most suitable, either with a chamber or with a breathing appliance. The difficulties of the determination of the respiratory quotient by the chamber method were pointed out by Carpenter (1915) who also came to the conclusion that the most logical method for the determination of the respiratory quotient with a breathing appliance was by the analysis

of expired air. Krogh and Lindhard (1920) made an analysis of the results obtained in the above publication (Carpenter, 1915) and chose the open circuit chamber apparatus for their extensive study of the respiratory exchange of muscular work. Benedict (1925) has recently stated that the open circuit arrangement with a chamber is the ideal method for the determination of the respiratory quotient.

The method of determining the total gaseous exchange during muscular activity and recovery and then calculating the respiratory quotient of the excess above the resting level has furnished a new point of attack on the problem of the source of energy for muscular work, but one must never lose sight of the fact that the excess respiratory quotient is a result of calculation and not the result of direct determination. It is not improbable that one may attach too much significance to this calculated ratio. All sorts of absurd ratios of excess gaseous exchange may be obtained if the suitable conditions are provided. An increase in carbon dioxide at rest with no increase in oxygen absorption will result in a calculated ratio of excess of infinity. So that one must be on guard against ascribing too much significance to ratios of excess gaseous exchange. A more correct perspective would be obtained from a study of the changes in total metabolism and total proportion of nutrients than from the metabolism and nutrients of the excess gaseous exchange due to muscular activity.

Various interpretations have been given to the results obtained by the different authors. It is evident that the proof is not adequate that carbohydrate is used exclusively in muscular activity of man. On the other hand, it does not seem evident that the nutrients used during muscular activity are used in the same proportion as they are during rest. Some of the experiments point in this direction but a general assessment of all of the results indicates that under certain conditions the proportion of carbohydrate used is greater during muscular activity than during rest. With very light moderate work of short duration there seems to be but little difference between the mixture metabolized during rest and that burned during work. On the contrary, when the work is increased, particularly in intensity, there is a general tendency toward an increase in the proportion of carbohydrate catabolized. When the work is continued and is more intense, the available carbohydrate seems to become diminished and then there is a call which results in a catabolism of fat with a lowering of the respiratory quotient. This catabolism is not necessarily a transformation of fat to carbohydrate with a subsequent catabolism of the latter.

The reviewer's hypothesis with regard to the fuel of muscular activity

is as follows: It would seem as though with extremely light work the available metabolites which result from the mixture of nutrients at rest were first used. These may be conceived of as small molecules from carbohydrate, protein, and fat. When the metabolites from fat which are easily oxidized are disposed of, the carbohydrate would be drawn upon, because this may be the more easily metabolized. Subsequently the available type of carbohydrate becomes diminished and then there is a diminishing amount of carbohydrate metabolized with an increasing amount of fat. It seems to the reviewer that some sort of combination, such as the above, may explain the lack of change in the respiratory quotient when the work is very moderate, the increasing carbohydrate utilized when the work is increased, and the drawing upon fat when the work is continued. This conception is somewhat like that of Bock and associates (1928) except that there is no implication of conversion of fat to carbohydrate. Such an hypothesis does not, however, explain the extraordinarily high quotients found with very intense work by Best, Furusawa, and Ridout. In fact, they are unexplainable at the present time.

In connection with recent work upon the source of energy for muscular activity very little or no consideration has been given to the rôle of ethyl alcohol in muscular activity. The literature on this subject is very conflicting, so that the question as to whether the energy of alcohol is convertible in muscular activity in the animal organism is by no means settled. It would seem that it would be worth while to reinvestigate the subject with present methods of study and points of view (excess respiratory quotient, formation and disappearance of lactic acid). It is evident that, if the availability of the energy of alcohol for the performance of muscular work is demonstrated, recent theories and conceptions of the chemistry of the metabolism of muscular activity would have to be revised. The use of ethyl alcohol as compared with simple sugars would also lead to evidence as to whether the size and availability of the molecules of nutrients or metabolites were correlated with their availability as sources of energy for muscular work.

It does not seem as though lactic acid were an essential member in the chain, although there is not the slightest doubt that lactic acid is formed in large amounts in muscular work, but it has been shown by several workers (Martin, Field, and Hall, 1929; Gollwitzer-Meier and Simonson, 1929) that the oxygen recovery curve and lactic acid disappearances are not parallel. The lactic acid remains a long time after the oxygen absorption has reached the pre-work level. Also, attention must be called to the fact

that the lactic acid-glycogen cycle exists during work as well as during recovery and that the liver is removing lactic acid from the blood even during muscular work (Himwich, Koskoff, and Nahum, 1930).

The studies of Eggleton and Eggleton (1927a, 1927b, 1928) on "phosphagen," the separation and identification of phosphocreatine by Fiske and Subbarrow (1927, 1929), and the investigations of Lundsgaard (1930a, 1930b) on muscular contraction without lactic acid formation have shifted the interest in the chemistry of muscular activity for the time being to another point of attack, and have led to questioning of the rôle of lactic acid in the chemical and energy transformations in muscles. However, not all of the adherents to the lactic acid theory have lost their faith, as Hill (1931) has recently expressed the opinion that lactic acid will "come back" if for no other reason than because of the far greater energy at its disposal than that of phosphagen. As stated at the beginning of this review, from time to time new investigations shift our interest. Will they remove completely the older conceptions and the older methods of attack or will they supplement, amplify, and explain results obtained?

T. M. C.

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